Pulmonary

1452 Prognostic Role of EGFR in Lung Adenocarcinoma Utilizing PCR Based Screening

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Background: Epidermal Growth Factor Receptor EGFR expression in lung cancer and its utility in predicting anti-EGFR responses are areas of intense research. Current studies analyzing the prognostic value of EGFR expression in lung tumors give conflicting results, as most research focuses on over-expression and not specific mutations. Recently identified tyrosine kinase domain EGFR mutations exons 18-21, have been found to confer increased EGFR inhibitor sensitivity to tumors with these mutations. Two of these mutations, a short exon 19 frame deletion and a exon 21 point mutation, account for roughly 90% of all mutations. In experimental models with these mutations these inhibitors induce apoptosis, reducing tumor growth. Since lung adenocarcinoma is a leading cause of cancer deaths, a screening test for these mutations could have value in the treatment of pulmonary adenocarcinoma.

Design: We examined 60 paraffin embedded sections cancers; 10 squamous cell, 10 small cell, 20 poorly differentiated, 20 well to moderate differentiated, and 10 broncho-alveolar (BA) malignancies. EGFR expression was determined by IHC via Dako EGFR PharmDX K. FISH using labeled DNA probes for centromere 7 and EGFR were used to examine EGFR expression and PCR was used to screen for exons 18-21 EGFR mutations.

Results: IHC EGFR protein expression analysis revealed the following; 4 of 5 cases of well–differentiated BA adenocarcinoma had strong staining. FISH analysis of 2 IHC positive cases were abnormal, with 3-8 EGFR copies /tumor cell. In moderately differentiated adenocarcinoma, 2 IHC positive cases examined by FISH had increased EGFR copies, 7 of 20 cases of moderately differentiated adenocarcinoma had IHC EGFR protein expression. Preliminary FISH analysis showed of these 3 positive cases had abnormal FISH assays, and 5 of 10 cases of poorly differentiated adenocarcinoma were positive for EGFR protein expression by IHC, with FISH analysis showing 2 positive cases with abnormal FISH.

Conclusions: We found a 100% concordance between IHC and FISH assay for BA and moderately differentiated adenocarcinomas, a 50% concordance for poorly differentiated adenocarcinoma. Analysis of these observations by PCR currently is in progress. A rapid and sensitive screening tool facilitating pulmonary adenocarcinoma diagnosis, directing therapeutic intervention would be valuable. Our data demonstrates that IHC, PCR, and FISH combined may have value in identifying patients with EGFR mutations that could benefit from EGFR inhibitors.

1453 Expression of Activated (pSTAT-3) and Inactivated STAT-3, in Non Small Cell Lung Carcinoma (NSCLC): Possible Role for Adjuvant Chemotherapeutic Intervention

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Background: Signal Transducer and Activator of Transcription 3 (STAT-3) is a point of convergence for numerous oncogenic signaling pathways and is constitutively activated at 50 to 90% frequency in diverse human cancers. A critical role of STAT-3 in tumor cell *survival*, proliferation, angiogenesis, metastasis and immune evasion has been demonstrated. The speculation is that by blocking pSTAT-3, it can potentiate the action of chemotherapeutic agents via increase in apoptosis. There is an unmet need to develop new treatment modalities for lung cancer. Recently it was shown that the inactive form of STAT-3 (cytoplasmic) is involved in cell motility and possibly in carcinogenesis. We evaluate the expression of both STAT-3s and proliferation marker MIB-1 in NSCLC.

Design: Tissue microarrays with triplicates of 86 archival cases of NSCLC were utilized. There were 17 Squamous cell CAs, 56 AdenoCAs and 13 large cell CA (LCCA). 75 were smokers and 9 were current non smokers and in 2 unknown. Age range was between 39-82 yrs. M:F were 1.6:1. In all cases Follow up was >5yrs. Immunohistochemical stains were performed as follows: STAT-3 and pSTAT-3 (Cell Signaling Technology, Inc., Beverly, MA; dil. 1:100), and MIB-1 (DakoCytomation, Carpinteria, CA; 1:100). The staining was interpreted as follows: STAT-3—nuclear and cytoplasmic; pSTAT-3—nuclear; and MIB-1—nuclear. Percent positivity of tumor cells was recorded on a scale 0-3(1=<33%, 2=34-66%, 3=>66%). Non-neoplastic lung tissues were used as controls.

Results: Both forms of STAT-3 were present in endothelium of vessels, stromal cells, and sparingly in bronchial epithelium in controls. In contrast strong expression for both forms was seen in morphologically normal bronchial epithelium and Type II pneumocytes adjacent to tumor. STAT 3 was also seen in inflammatory cells next to tumor. STAT-3 was expressed in 65% (>2+ in 63%) and pSTAT in 44% (>2+ in 28%) of NSCLC. There was no correlation with MIB-1, Tumor Type, Age, Sex, Smoking Status, Stage and Grade of Cancer, and survival with either STAT-3.

Conclusions: Contrary to other authors, there is no correlation with current non smokers/never smokers with both forms of STAT-3. Presence of STAT-3 on inflammatory cells may contribute to their motility/carcinogenesis. STAT-3 and pSTAT-3 are up regulated in a high percentage of NSCLC providing basis for therapeutic intervention.

1454 Expression of Activated (pSTAT-3) and Inactivated STAT-3, in Malignant Mesothelioma (MM): Possible Role for Chemotherapeutic Intervention

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Background: Signal Transducer and Activator of Transcription 3 (STAT-3) is a point of convergence for numerous oncogenic signaling pathways and is constitutively-activated at 50 to 90% frequency in diverse human cancers, including common solid tumors. A critical role of STAT-3 in tumor cell *survival*, proliferation, angiogenesis, metastasis and immune evasion has been demonstrated. The speculation is that by blocking pSTAT-3, it can potentiate the action of chemotherapeutic agents via increase in apoptosis. STAT-3 expression has not been studied in MM and there is an unmet need to develop new treatment modalities for MM. Recently it has been shown that the inactive form of STAT-3 (cytoplasmic) is involved in cell motility and may also be important in carcinogenesis. The objective is to study the expression STAT-3, pSTAT-3 in conjunction with proliferation marker MIB-1 in MM.

Design: Tissue microarrays with triplicates of 44 archival cases of MM were utilized. There were 22 epithelioid MM, 9 Biphasic MM and 13 Sarcomatous MM. Immunohistochemical stains were performed on all cases as follows: STAT-3 and pSTAT-3 (Cell Signaling Technology, Inc., Beverly, MA; dil. 1:100), and MIB-1 (DakoCytomation, Carpinteria, CA; 1:100). The staining was interpreted as follows: STAT-3—nuclear and cytoplasmic; pSTAT-3—nuclear; and MIB-1—nuclear. Percent positivity of tumor cells was recorded on a scale 0-3 (1=<33%, 2=34-66%, 3=>66%). Non-neoplastic lung tissues were used as controls.

Results: Both forms of STAT were present in endothelium of vessels, stromal cells, and in normal mesothelium in non neoplastic controls. In addition, STAT 3 was seen predominantly in inflammatory cells next to tumor. STAT-3 was strongly expressed in 82% of epithelioid MM, 100% of biphasic MM and 62% of sarcomatous MM. pSTAT was expressed in 45% of epithelioid MM, 56% of biphasic MM and 15% of sarcomatous MM. There was no correlation with MIB-1,

Conclusions: Both forms of STAT-3 are up regulated in a high percentage of MM providing basis for therapeutic intervention

1455 Pathological and EGF-r Analysis of Tumor Samples from Patients with Adenocarcinomas with BAC Features (BAC) Treated by Gefitinib. IFCT0401-Bio Trial

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Background: Pneumonic -type adenocarcinoma is often a bronchioloalveolar carcinoma (BAC) variant in the 2004 WHO classification. A french prospective multicentric phase II trial (IFCT0401) evaluated gefitinib as first line treatment in non-resectable P-ADC. Tissue samples were collected for central pathological review and molecular analysis in attempt to determine if an association existed between disease control (DC) and biological markers.

Design: Histologic types were classified according to the 2004 WHO classification as BAC variants and ADC, other types and as non-mucinous or mucinous/mixte. Immunohistochemistry was performed using antibodies against the following markers : TTF1, Ki67, CK 7, CK20, P AKT, cerbB2 and EGFR. Polysomy/amplification was examined for cerbB2, and EGFR. EGFR 18-21 and K-ras 1 exons were amplified and sequenced.

Results: A tissue specimen was collected from 67 of the 88 eligible participants, among which 34 were from surgical resection; this subgroup did not differ from the overall trial population in terms of sex ratio(0.53 vs 0.56), proportion of non-smokers (32 vs 40 %) and DC rate (35 vs 29 %). Results described herein were from these 34 surgical specimens. 27 were BAC variants (79 %) and 7 ADC other types. Of the 34, 20 were non-mucinous and 14 mucinous. TTF1, Ki67, PAKT, cerbB2 as well as EGFR expression did not differ between BAC variants and ADC other types. TTF1 and EGFR scores of expression were higher in non-mucinous than in mucinous P-ADC. DC with gefitinib was significantly associated with non-mucinous subtype (p=0.009) and EGFR mutations (p=0.01), moreover combined with polysomy or amplification (p=0.001). There was no relation with other markers. K-ras exon 1 codon 12 mutation was found in 9 tumors of which 8 progressed on gefitinib. Polysomy of EGFR was seen in 3 tumors. One of which also contained EGFR mutation (exon-19 deletion). Both were controlled by gefitinib.

Conclusions: Among patients with ADC with BAC features who received gefitinib, non-mucinous subtype, and EGFR polysomy and/or mutation may have improved DC, while K-ras mutations seem associated with disease progression.

1456 Histologic Assessment and Prognostic Factors of Malignant Pleural Mesothelioma Treated with Extrapleural Pneumonectomy

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Background: Pleural malignant mesothelioma is a tumor with poor prognosis. It has been stated in the past that the epithelial type has better prognosis than the other histological types. We have evaluated different prognostic factors with emphasis on histological subtype on patients who have undergone extrapleural pneumonectomy (EEP).

Design: Fifty-six extrapleural pneumonectomies (EEP) were evaluated over a period of 18 years (1986-2004). All available clinical information including tumor stage was analyzed. All tumors were classified according to WHO classification and correlated with the histological type of the diagnostic procedure (biopsy or cytology). Kappa statistics were used to assess the agreement between histology of diagnostic procedure and EEP.

Uni- and multivariate analyses were performed to evaluate prognostic significance of the histological type. Clinical parameters were correlated with disease specific and recurrence free survival.
Results: The patients are 50 men and 6 women between the ages of 37 and 80 (median age: 60 years). Clinical staging was obtained in 54 patients: **Stage I**: one patient; **Stage II**: 6 patients; **Stage III:** 33 patients; **Stage IV:** 14 patients. Six patients received pre-operative chemotherapy while 33 patients received post-operative adjuvant treatment. Forty-six and 44% respectively of epithelial and sarcomatous mesotheliomas on initial biopsy were reclassified after EEP as biphasic tumors (p-value: 0.0001). Uni and multivariate analyses show significant association between histological type of diagnostic procedure and disease specific survival; post-operative treatment with disease specific survival, and recurrence free survival. However, the histological type after EEP was not significantly associated with survival. Age, gender, stage, side or preoperative treatment did not affect disease specific survival or recurrence free survival.
Conclusions: At least 40% of the interpretation of the histological type on biopsy specimens may change after EEP. The increase in the number of biphasic tumors and the lack of association of histological type of the EEP with disease specific or recurrence free survival suggests the possibility that some biphasic tumors may behave either as epithelial or sarcomatous neoplasms. Better outcome was shown in patients with adjuvant treatment after EEP.

1457 Combined Cytopathologic and Radiologic Diagnosis of Adenocarcinoma with Bronchioloalveolar Alveolar Features (ADC-BAC) Is Highly Predictive of Subsequent Histopathologic Diagnosis and Clinical Outcome

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Background: Non-mucinous bronchioloalveolar adenocarcinoma (BAC) displays characteristic cytopathologic features in fine needle aspiration biopsies (FNAB) that, however overlap those of atypical adenomatous hyperplasia and mixed adenocarcinomas with a BAC component. Radiographically BAC is associated with single or multifocal nodules, ground glass opacity (GGO), and central solid component in mixed adenocarcinomas. The purpose of this study is to determine the accuracy of ADC-BAC diagnosed by FNAB for predicting the histopathologic diagnosis and patient outcome.
Design: FNAB with cytologic features of ADC-BAC (sheets of cells with pale chromatin, inconspicuous nucleoli, nuclear grooves, intranuclear cytoplasmic inclusions and background histiocytes) were prospectively identified. Corresponding surgical excision specimens were reviewed and categorized as BAC pattern with ≦0.5 central scar (NMBAC), mixed adenocarcinomas with any BAC component and >0.5 cm scar (ACA), and mixed adenocarcinomas with no BAC component (non-BAC). Cytopathologic findings were correlated with radiologic and histopathologic findings and clinical follow-up.
Results: 39 lung tumors from 32 patients were included. ACA tumors had median BAC component of 50%, and median scar size of 0.9 cm. 2 of 21 ACA had a scar > 1.5 cm. 3 of 7 NMBAC were without a central scar. Median follow-up time was 40 months. 14 of 15 (93.3%) GGO tumors had a BAC component, all 14 of the patients had no evidence of disease, 2 of the 14 had multifocal disease, and none of the 14 tumors had adverse prognostic indicators such as tumor recurrence, metastasis, tumor size greater than 3 cm, central scar larger than 1.5 cm, vascular or pleural invasion.
Conclusions: Combined cytopathologic and radiologic criteria can accurately diagnose adenocarcinomas with a BAC component and correlate with favorable outcome.

Histopathologic diagnoses and clinicial outcome

	n	AWD	NED
NMBAC	7 (18%)	0	7 (100%)
ACA	21 (54%)	1 (4.7%)*	19*(90.4%)
non-BAC	11 (28%)	3 (27.3%)	8 (72.7%)

AWD alive with or died of disease, NED no evidence of disease. * One patient lost to follow up.

Radiologic findings

	NMBAC	ACA	NON-BAC
Ground glass opacity	5 (83.3%)	9 (60.0%)	1 (11.1%)
Part-solid nodule	1 (16.7%)	5 (33.3%)	2 (22.2%)
Solid nodule	0	1 (6.7%)	6 (66.7%)
Total	6 (100%)	15 (100%)	9 (100%)

1458 Large Cell Neuroendocrine Carcinoma – A Distinct Expression Profile Relative to Large Cell Undifferentiated Carcinoma

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Background: Large cell neuroendocrine carcinoma (LCNEC) is classified by the WHO as a large cell lung carcinoma, rather than a high grade neuroendocrine carcinoma. We have recently shown that survival for patients with LCNEC was significantly lower than in patients with large cell undifferentiated carcinoma (LCUC). The objective of the study was to compare the gene expression profiles of these two entities with the goal of finding clinically useful molecular markers as well as an expression profile signature that could be applied to other NSCLC for prognostic information.
Design: Snap frozen tumor tissue from cases of LCNEC (n=10) and LCUC (n=10) were collected under IRB approval, sectioned, and histologically reviewed to confirm neoplastic cell content, and then utilized for RNA isolation. Biotinylated cRNA targets were generated from each RNA sample, and these in turn were hybridized to Affymetrix U133Plus2 human GeneChip® microarrays using standard protocols. Signal data from approximately 54,000 probe pair sequences was normalized using the Affymetrix MAS5 statistical algorithm and filtered for non-detectable signals. Data was annotated using the Siteman Cancer Center Bioinformatics Core and analysis and visualization was performed using DecisionSite for Functional Genomics (Spotfire, Inc) software.
Results: After unsupervised hierarchical clustering, all LCUC tumors clustered together and all but one LCNEC formed a second cluster. We identified a set of 63 transcripts whose expression was statistically different between the two tumor groups (differences ranging from 5 to 38 fold increased expression in LCNEC). Among these were *NCAM1*, *FZD3 or* Frizzled homologue 3, *SNCAIP or Synphilin-1, and PTPRO or Protein tyrosine phosphatase receptor type O or GLEPP 1*. Current work is focused on identifying aberrant signaling pathways in these tumors based upon their gene expression profiles, and validating patterns of gene expression identified in this study using a larger, independent cohort of paraffin embedded cases via immunohistochemistry and quantitative RT-PCR.
Conclusions: We have demonstrated a robust molecular signature that distinguishes these two variants of NSCLC. In the future, some or many of the genes which contribute to this signature may become important biomarkers or therapeutic targets for the specific clinical management of LCNEC.

1459 C4d Deposition in Lung Allograft Biopsies: Detection by Immunohistochemistry, Correlation with Serum Anti-Donor Antibodies, Histology and Patient Outcome

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Background: Humorally mediated rejection is a well described and often diagnosed complication of cardiac and renal transplantation. However, it remains a poorly characterized entity in lung transplantation. Furthermore, among the few published studies there have been conflicting results, particulary in relating complement deposistion and anti-donor serum antibody titers. The aim of this study is to investigate C4d deposition in relation to anti-donor antibodies, biopsy morphology and patient outcome.
Design: A total of 94 consecutive lung allograft biopsies, from 85 of patients were collected under IRB approval from our departmental files between 2004 and 2006. Immunohistochemistry for C4d was performed on 4 micron thick sections using standard protocols. Biopsies were examined blindly by two pathologists and staining for C4d was graded as negative, focal, or diffusely positive based on capillary endothelial cell staining. Cardiac allograft biopsies with known humoral rejection were used for positive controls. Results were then correlated with biopsy morphology and the presence of anti-donor serum antibodies.
Results: Strong capillary and endothelial deposition of C4d was seen in 10.6% (n=10). Focal C4d positivity was seen in 20.2% (n=19), and 69.2% (n=65) were negative. Among the 10 patients with strong C4d deposition, 50 % showed rejection-related biopsy findings including chronic rejection (n=2), acute cellular rejection (n=1), and capillaritis (n=2). Anti-donor antibodies were present in two patients. On biopsy one patient showed hemorrhagic necrosis with neutrophilic vasculitis, and had anti-donor antibodies at the time of implantation. This patient suffered allograft loss. The second patient showed capillaritis two weeks post transplant and responded to plasmapheresis.
Conclusions: Vascular C4d can be demonstrated in a minority of lung allografts. Only a subset of those positive cases will show classic morphologic findings of acute humoral rejection. On the other hand, C4d deposition may be present with other forms of rejection, including chronic rejection, where its importance is yet to be elucidated.

1460 Histopathologic Characteristics of Lung Adenocarcinomas with Loss of MGMT Expression

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Background: O^6-methylguanine-DNA-methyltransferase (MGMT) encodes O^6-alkylguanine DNA alkyltransferase (AGT), a DNA repair protein that maintains genomic stability by removing alkylating lesions at position O^6 of guanine. Loss of MGMT expression has been shown to increase the risk of carcinogenesis and has been associated with increased sensitivity to treatment with alkylating agents. We evaluated MGMT expression in pulmonary adenocarcinomas and correlated the findings with tumor differentiation and morphology.
Design: We studied 140 consecutive patients with lung adenocarcinomas treated with surgery at Brigham and Women's Hospital between January 1997 and December 1999. Histopathologic characteristics in H&E stained slides were recorded according to WHO criteria without access to clinical data. MGMT expression was assessed by immunohistochemistry using staining of intratumoral lymphocytes as a positive control. MGMT protein expression was assessed semiquantitatively on the basis of the most intense staining of tumor cells and assigned to one of four categories: no staining/loss of expression (score=0); minimal staining (score=1); staining intensity equivalent to that of intratumoral lymphocytes (score=2); strong staining intensity (score=3). MGMT expression was considered focally lost only in heterogeneous tumors in which one component had complete loss of expression.
Results: Twenty-eight (20%) lung adenocarcinomas were well-differentiated, 71 (51%) were moderately-differentiated, and 41 (29%) were poorly-differentiated. Absence of MGMT expression was complete in 11 (7.9%) lung adenocarcinomas and focal in 2 (1.4%). MGMT expression correlated with tumor differentiation (p=0.03) and histologic subtype (p=0.004). Poorly-differentiated adenocarcinomas had lower MGMT expression than moderately-differentiated and well-differentiated adenocarcinomas. Of note, none of the lung adenocarcinomas with loss of MGMT expression were well differentiated.
Conclusions: Our study defines the histopathologic features of a molecular subtype of lung adenocarcinomas characterized by loss of MGMT expression. Since loss of MGMT expression is associated with increased sensitivity to treatment with alkylating agents, our results suggest that identifying lung adenocarcinomas with MGMT silencing may offer a novel therapeutic alternative in this subset of patients.

1461 Retinoic Acid Receptor Beta Expression in Non Small Cell Lung Carcinomas

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Background: Retinoids, analogues of vitamin A, are required for the normal lung development, growth and differentiation. They are also able to reverse premalignant lesions and prevent second primary tumors in some patients with non-small-cell lung cancer (NSCLC). These effects are believed to result from modulation of cell growth, differentiation, or apoptosis (programmed cell death). Some studies have demonstrated RAR alpha expression in more than 95% of the NSCLC specimens whereas RAR beta expression is detected in only 42% of NSCLC. RAR beta expression has been used as a prognostic indicator in stage I non-small-cell lung cancer. When certain retinoid receptors in the cell nucleus are suppressed, abnormal activity may result that could enhance cancer development.

Design: Tissue microarrays containing triplicate punch samples of 340 NSCLCs with 5 years or more follow-up were immunostained with antisera for retinoic acid receptor beta (Abcam, 1:25, Dual Envision detection method). Staining intensities were graded as 0, 1+ (weak), 2+ (moderate), or 3+ (strong) and the percentages of tumor cells staining were approximated to the nearest 10%. For each tumor, mean values for staining intensity and percentage were calculated, and used to derive an overall score of 0-3. Correlations with survival and histologic type were analyzed using Log-Rank test (Cox-Mantel), Spearman rank correlation and Kaplan-Meier survival table.

Results: 299 NSCLC were stages I and II, and 61 were stages III and IV. 82% of NSCLC sections were positive for RAR-beta including all cell types however, mucinous adenocarcinomas were consistently negative regardless of the stage. In our cases, expression of RAR-beta did not correlate with survival. These results are different from those described by others however, RAR-beta expression is expressed in several isoforms due to splicing and usage of alternative promoters. The antibody used by us most likely detects isoforms not seen in other studies.

Conclusions: RAR-beta is widely expressed in NSCLCs and variations in its expression patterns show no relationship to prognosis regardless of other factors such as sex and histologic type.

1462 Multimarker Immunohistochemistry of Non-Small Cell Lung Carcinomas: Correlation with *EGFR* Mutation and Response to Treatment

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Background: Non-small cell lung carcinomas (NSCLCs) with mutations in the epidermal growth factor receptor *(EGFR)* respond to therapy with tyrosine kinase inhibitors (TKIs, gefitinib, erlotinib). However, *EGFR* mutation analysis is not widely available. Immunohistochemistry (IHC) is a more accessible technique, but has not been shown to predict response to TKIs in NSCLCs. Since EGFR is activated by mutations in NSCLCs, a panel of antibodies to proteins downstream of EGFR were tested, as well as EGFR and several differentiation markers, in a set of NSCLCs with known sequence and response.

Design: 69 cases of NSCLC (65 adenocarcinomas, 4 large cell carcinomas) were reviewed. IHC was performed with antibodies to EGFR, phosphorylated (p)EGFR, pAKT, pSTAT3, CK7, CK20, TTF-1, chromogranin, and surfactant protein A. Each slide was reviewed by two pathologists for staining pattern, % of tumor cells staining, and intensity (0-4+) . For each marker, a staining index was calculated by multiplying % positivity x intensity, and compared with outcome and *EGFR* sequence.

Results: The strongest predictor of response was *EGFR* mutation ($p<0.0001$), but several IHC markers also predicted response and/or *EGFR* mutation. High cytoplasmic EGFR, membranous EGFR, and membranous pEGFR were predictors of response ($p=0.001$, 0.003, 0.025), and high cytoplasmic EGFR predicted mutation ($p=0.03$). High cytoplasmic pAKT predicted mutation ($p=0.034$), and high membranous pSTAT3 predicted response ($p=0.019$). Interestingly, high TTF-1, a lung differentiation marker, predicted mutation ($p=0.008$) and response ($p=0.002$). Other markers did not show significant associations with response or mutation.

Conclusions: The association between *EGFR* mutation and response to TKIs in NSCLCs was confirmed. IHC, a less costly and more widely available technique, showed associations between several immunostains (EGFR, pEGFR, pAKT, pSTAT3, and TTF1) and response and/or *EGFR* mutation. These differences in IHC are suggestive of biologic differences, may be useful in prediction of *EGFR* mutation status and response to TKIs, and warrant further study.

1463 Correlation of Papillary and Bronchioloalveolar Patterns in Lung Adenocarcinoma with *EGFR* Mutation and Response to Tyrosine Kinase Inhibitors

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Background: Mutations in the epidermal growth factor receptor (*EGFR)* predict response of lung adenocarcinoma to treatment with tyrosine kinase inhibitors (TKIs, gefitinib, erlotinib). Since *EGFR* sequence analysis is not widely available, histologic features correlating with *EGFR* mutation and/or response to TKIs may prove valuable. Correlation between *EGFR* mutation and bronchioloalveolar carcinoma (BAC) has been shown, with conflicting data in rare reports on papillary adenocarcinomas.

Design: Representative H&E stained sections of 53 lung adenocarcinomas treated with TKIs were reviewed by two pathologists. Adenocacinoma subtypes and achitectural patterns were correlated with *EGFR* sequence and clinical response to TKIs.

Results: Of the 53 cases, 4 were BACs, all of which were non-mucinous, and all with *EGFR* mutations. 7 cases had a prominent papillary pattern (5 papillary adenocarcinomas, 2 BACs with papillary pattern), all of which had *EGFR* mutations; all 4 of these with clinical data responded to TKIs. A bronchioloalveolar pattern was seen in 11 of 44 acinar adenocarcinomas, but was not associated wtih EGFR mutation or response to TKIs.

[Table1]: Lung adenocarcinomas vs EGFR mutation and response to TKIs

	EGFR mutation		Response to TKIs		
	Positive	Negative	Response	Stable	Progress
Acinar adenocarcinoma	23	10	22	10	6
Acinar adenocarcinoma with prominent bronchioloalveolar pattern	6	5	4	2	2
BAC without papillary pattern	2	0			
BAC with papillary pattern	2				
Papillary adenocarcinoma	5	0	4		
Total	38	15	30	12	8

Conclusions: The reported association between *EGFR* mutation and BAC subtype was confirmed, but no association was shown between bronchioloalveolar pattern in acinar adenocarcinoma and *EGFR* mutation/response to TKIs. In contrast, all cases with prominent papillary features -papillary adenocarcinomas and BACs with papillary pattern- had *EGFR* mutations and responded to TKIs. Larger studies are warranted to confirm the significance of papillary pattern in prediction of *EGFR* mutation and/or response to TKIs.

1464 IRS-1 and Cyclin B1 Are Associated with Decreased Survival in Stage I Lung Adenocarcinoma

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Background: Predictors of survival in non-small cell carcinoma, especially in Stage I disease, may help guide therapeutic decision making. Previously, using gene expression profiling of biopsy specimens, we identified 9 genes associated with survival. To validate this finding, we performed immunohistochemistry in an independent cohort of 180 patients.

Design: Four of the 9 genes (IRS1, Cyclin B1, TGF-B, and FHL2) for which commercial antibodies were available were selected for immunohistochemistry studies. Tissue microarrays (TMA) were constructed containing cores from lung adenocarcinomas of 180 patients with a minimum of five year clinical follow-up after surgical resection at Columbia Presbyterian between 1997-2000. TMA immunostaining was scored from 0-3 for intensity and for percent positive staining tumor cells.

Results: Survival data was available for 168 of 180 patients (93%). Increased IRS-1, CCNB1 and TGF-B immunostaining was correlated with increased 5 year mortality (correlation co-efficients: .319 $p<.0001$; .317 $p<.0001$; .178 $p<.023$, respectively). Cox regression analysis indicated IRS-1 and CCNB1 were associated with decreased survival time (Hazard Ratio 2.1, $p<.0001$ and 2.88, p.0001, respectively). Within the subgroup of Stage 1 patients (n=120), IRS1 and CCNB1 staining was correlated with increased 5 year mortality (.277 $p<.001$,.242 $p<.003$), and decreased survival time (HR 2.0 $p<.002$, HR 3.8 $p<.001$). Within the subgroup of Stage 1a patients (n=73), IRS-1 and CCNB1 remained significantly associated with 5 year mortality (.376, $p<.003$ and .260, $p<.003$), and decreased survival time (HR 2.8 $p<.002$, 4.2 $p<.005$ respectively).

Conclusions: Immunohistochemistry validated the prognostic significance of gene signatures of lung cancer outcome. Increased immunostaining for IRS1 and cyclin B1 was associated with increased 5-year mortality and decreased 5 year survival time for Stage I patients and for Stage IA lung adenocarcinoma patients.

1465 The Histologic Assessment of Pulmonary Non-Small Cell Carcinoma after Neoadjuvant Therapy

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Background: Neoadjuvant therapy using chemotherapy and/or radiation therapy for advanced non-small cell carcinoma of the lung has been shown in some studies to increase survival when compared to other treatments. Although the histologic changes seen in other previously treated tumors (e.g., rectal adenocarcinoma) have been well-described, those seen in pulmonary specimens after neoadjuvant therapy have been characterized incompletely. We review our experience with consecutively resected non-small cell carcinomas of the lung that have received neoadjuvant therapy.

Design: All specimens over a two year period from patients who received neoadjuvant therapy for advanced non-small cell carcinoma were reviewed. Original biopsies were reviewed and assessed for the histologic tumor type and degree of differentiation. Surgical specimens were reviewed and assessed for histologic tumor type, degree of differentiation, percent necrosis, percent and characterization of fibrosis, cytologic changes reflecting treatment effect, inflammatory changes and other findings. Response was categorized as complete (no remaining tumor seen), nearly complete (>90% necrosis and fibrosis), partial (25-90% necrosis and fibrosis), and limited (<25% necrosis and fibrosis).

Results: Fifty-one cases were reviewed. Of these, 7 showed complete response, 13 showed near complete response, 17 showed partial response and 14 showed limited response. The proportions of fibrosis and necrosis were extremely variable from case to case. Fibrosis was characterized by loose, pale-staining, fibroelastic tissue. Intermixed mild to moderate chronic inflammation was present, frequently with interspersed foreign body cholesterol granulomas corresponding to the amount of necrosis. Surrounding lung tissue typically showed mixed inflammation with atypical pneumocyte hyperplasia, but distinct therapy-induced cytologic atypia was seen only in small foci of neoplastic cells. Complete and near complete responders were more likely to have had poorly differentiated lesions in the original biopsy than partial and limited responders.

Conclusions: We conclude that the histology seen in pulmonary specimens after neoadjuvant therapy for non-small cell carcinoma is reproducible but relatively non-specific. It remains to be seen whether any changes seen within the biopsy or resection specimens will correlate with survival data.

1466 Evidence of Epithelial/Mesenchymal Transition in Idiopathic Pulmonary Fibrosis

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Background: Fibroblastic foci are thought to represent a lesion central to the pathogenesis of idiopathic pulmonary fibrosis (IPF). However, the etiology of fibroblastic foci in IPF is unclear. Recent experimental data suggests that the conversion of alveolar epithelial cells (AECs) into fibroblasts can occur in response to transforming growth factor (TGF)-b, and this process may play a role in IPF (Willis et al. 2005, Kasai et al. 2005). We therefore sought further evidence of epithelial/mesenchymal transitions in giving rise to fibroblastic foci.

Design: We identified 11 IPF patients with lung biopsy specimens and performed immunohistochemical stains for thyroid transcription factor (TTF)-1 and surfactant protein (SP)-A, which are normally expressed by AECs. We then analyzed expression of these markers within fibroblastic foci.

Results: In all 11 lung biopsy specimens we examined, we were able to identify cells within fibroblastic foci that showed expression of either TTF-1 or SP-A. These cells typically were morphologically similar to fibroblasts, and did not appear to be AECs.

Conclusions: Our data support a role for EMT in the etiology of FF in IPF, consistent with data from prior studies (Willis et al. 2005). Importantly, we examined lung biopsy specimens from 11 patients, the largest sample size yet reported. Dual staining to co-localize additional epithelial and mesenchymal markers will also lead to a better understanding of the pathogenesis of IPF and other interstitial lung diseases.

1467 Can Pathologists Agree on What Constitutes Visceral Pleural Involvement by Non-Small Cell Lung Carcinoma? An Internet-Based Assessment of International Current Practices

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Background: Visceral pleural involvement (VPI) upstages non-small cell lung carcinomas (NSCLC) that otherwise only meet pT1 criteria to pT2. Despite its prognostic importance, the AJCC provides no guidelines on what constitutes VPI. Penetration of tumor through the visceral pleural elastic layer (VPEL) has been proposed as the minimum criterion necessary for VPI, but this has not been internationally accepted. To quantify the diagnostic variability among pathologists in assessing VPI, we examined responses to an international online quiz.

Design: The quiz consisted of 15 cases of NSCLC adjacent to or involving the visceral pleura. Each case had 2-4 images, including at least 1 EVG-stained section. Participants were asked to determine if VPI was present, absent, or indeterminate.

Results: Of 103 participants, 22 countries were represented, 84% were in academic practice, 43% had a subspecialty interest in pulmonary pathology, and 15% were housestaff. For the quiz cases, concordance ranged from 37-93% (avg. 73%). The highest concordance was observed in cases where VPEL penetration was unequivocally absent, which 90% of participants categorized as VPI absent, followed by cases with unequivocal extension beyond the VPEL, which 77% categorized as VPI present. The greatest discordance was found in cases with extensive elastosis and cases in which the VPEL was inconspicuous. The rate of discordance was higher among participants who did not have a subspecialty interest in pulmonary pathology. The frequency with which cases were categorized as VPI indeterminate ranged from 1-37% (avg. 11%).

Conclusions: Although this study showed relatively high interobserver agreement regarding the presence or absence of VPI, there is considerable diagnostic variability in cases with extensive pleural elastosis. While the majority of pathologists in this study considered penetration of the VPEL as necessary and also sufficient to categorize VPI as present, the formation of internationally recognized guidelines for assessing VPI by NSCLC is likely to improve diagnostic consensus.

1468 Thromboxane Synthase A2 Overexpression in Non-Small Cell Lung Carcinoma (NSCLC) and Its Correlation with Tumor Type and Grade

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Background: Thromboxane Synthase-A2 (TXS-A2) is a downstream enzyme in the prostaglandin biosynthetic pathway. Cyclooxygenase (COX) inhibitors, which block the synthesis of prostaglandins upstream, have been studied and shown to augment lung cancer therapy. We hereby present our evaluation of the overexpression of TSX-A2 in NSCLC by immunohistochemical methods.

Design: An electronic search of our Surgical Pathology database for non-small cell lung carcinoma yielded 25 cases. These included 11 cases of squamous cell carcinoma and 14 cases of adenocarcinoma. Representative slides were immunohistochemically stained for TSX-A2. The slides were evaluated for tumor grade, extent/intensity of tumor cell staining, and staining qualities of endothelial cells within and adjacent to tumor foci. The intensity of staining was recorded on a 1+ (scant) to 4+ (intense) scale.

Results: Eight cases of well-differentiated adenocarcinoma demonstrated high levels of TSX-A2, with 6 cases showing 4 + staining and 2 cases showing 3+ staining. Four cases of moderately-differentiated adenocarcinoma demonstrated variable staining intensity (1 case = 3+, 2 cases = 2+, 1 case = 1+). Two cases of poorly-differentiated adenocarcinoma had (1+) staining. Eleven squamous cell carcinomas were identified (well-differentiated = 1 case, moderately-differentiated = 9 cases, poorly-differentiated = 1 case). Of the moderately-differentiated cases, 5 cases showed 2+ staining while 4 cases showed 1+ staining. The single well-differentiated tumor showed 3+ staining, while the one poorly-differentiated tumor showed 2+ staining. In 19 of the 25 cases, the vessels in and adjacent to the tumors were evaluated for TSX-A2 staining. 15 of the 19 cases demonstrated 2+ to 3+ staining, which was more prominent than the staining observed within vessels remote from tumor foci.

Conclusions: The level of overexpression of TSX-A2 in NSCLC correlates with the histological type of carcinoma, exhibiting higher levels in adenocarcinoma and lower levels in squamous cell carcinoma. In general, the well-differentiated tumors demonstrated higher levels of TSX-A2 than poorly-differentiated lesions. The endothelial cells of the blood vessels within or adjacent to the tumors showed higher levels of expression than those away from the tumors. These results will need to be confirmed in larger studies and may demonstrate the utility of COX inhibitors in the treatment of NSCLC.

1469 Expression of Osteopontin in Tissue Microarray of 274 Non-Small Cell Lung Carcinomas: Relationship to Lymph Node Metastases

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Background: Despite recent advances in the treatment of non-small cell lung cancers (NSCLCs), the 5-year survival rate remains dismal due primarily to metastatic disease. Osteopontin (OPN) is a multifunctional cytokine and adhesion protein that has been implicated in carcinogenesis, tumor progression and metastasis of several human cancers including breast, colon, prostate, gastric and lung. OPN represents a logical candidate molecule to study for its relationship to metastasis in lung cancer. Therefore we evaluated the immunohistochemical expression of OPN in 274 NSLC and its relationship to lymph node metastasis.

Design: Tissue microarrays containing triplicate punch samples of 274 cases of NSCLCs were immunostained for osteopontin (1:100, Calbiochem) using standard avidin-biotin techniques. For each punch sample, the percentage of tumor cells staining was scored as positive if 5% or more cells had cytoplasmic staining and negative if 0-4% cells had cytoplasmic staining. For each tumor, mean values for staining were calculated and used for statistical analysis. Lymph node status based on standard TNM staging was collected for all cases (N= 0, 1, 2 or 3) and OPN expression analyzed in relation to lymph node status by the Fisher exact chi square test

Results: The OPN expression in NSCLC with lymph node metastasis (N = 1, 2 or 3) was significantly higher than that from tumors without metastasis ($p = 0.047$).

Conclusions: Overexpresssion of osteopontin is associated with increased risk of lymph node metastasis in NSCLC. Pharmacological manipulation of OPN may provide a novel therapeutic approach for the prevention and treatment of NSCLC lymph node metastasis.

1470 Immunohistochemical Staining Differences between Malignant Mesothelioma and Squamous Cell Carcinoma

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Background: Malignant mesothelioma (MM) and squamous cell carcinoma (SCC) can occasionally be difficult to differentiate on a pleural biopsy. Most of the current literature focuses on immunohistochemical markers to differentiate between adenocarcinoma and MM. The purpose of this study was to evaluate the immunoreactivity pattern of MM and SCC using a panel of mesothelial and epithelial markers.

Design: 25 cases of MM (18 epithelioid, 4 spindled and 3 biphasic subtypes) and 25 cases of SCC of the lung were retrieved with IRB approval. Tissue microarrays were made of 1mm cores of representative areas from paraffin blocks . Sections were stained with calretinin, D2-40, MOC 31, p63, WT-1, BG8, CK20, CK5/6, CK7 and TTF-1. The cases were scored based on intensity of immunoreactivity from 0 to 3+.

Results: Two epithelioid MM cases were excluded due to absence of tumor on microarray sections. Of the MM cases, 91% were positive for D2-40 (1+ to 3+), 87% for calretinin (1+ to 3+), 83% for CK7 (1+ to 3+), 74 % for WT-1 (nuclear, 1+ to 3+), 43% for CK5/6 and MOC31 (1+ to 3+), 17% for CK20 (1+), 13% for p63 (1+), and 9% for BG8 (1+). In contrast, 100% of the SCC were positive for p63 (2+ to 3+) and MOC31 (only one was 1+, all others 2+ to 3+), 96% for CK5/6 (1+ to 3+) and BG8 (1+ to 3+), 52% for calretinin (1+ to 2+), and both CK7 (2+ to 3+) and D2-40 (1+) were positive in 20%. Nuclear staining for WT-1 was negative in all SCCs. All MM and SCC cases were negative for TTF-1.

Conclusions: There was significant overlap in staining for calretinin and CK5/6 between MM and SCC. Mesotheliomas were more likely to be strongly positive for D2-40, WT-1 (nuclear), calretinin and CK7, and were negative or weakly positive for p63, MOC31, and BG8. Nuclear staining for WT-1 appeared to be the most specific marker for MM. SCC were more likely to be strongly positive for p63, MOC31 and BG8 and negative or weakly positive for D2-40. Thus a panel of immunohistochemical markers should be used to differentiate between MM and SCC of the lung. The results of this study emphasize the fact that the stains have to be interpreted carefully, both in regards to intensity and location.

Immunohistochemical Staining results of Maligant Mesothelioma and Squamous Cell Carcinoma

	MM (of 23 cases)	SCC (of 25 cases)
D2-40	21	5
Calretinin	20	13
WT-1	18	0
CK 7	17	5
CK 5/6	10	24
Moc 31	10	25
CK 20	4	0
p63	3	25
BG8	2	24
TTF-1	0	0

1471 Metagenomic Analysis of Pulmonary Bacterial Microflora in Cystic Fibrosis

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Background: Cystic fibrosis (CF) is characterized by progressive loss of lung function as a consequence of chronic inflammation of airways secondary to bacterial colonization. While a number of bacteria are known patholgens in CF, there is growing evidence that the lungs of CF patients may harbor numerous bacterial speicies and that interactions within this ecosystem may affect clinical course. However, the composition of this bacterial community is not well characterized. We therefore initiated work to identify bacterial speicies present in the lungs of CF patients by 16S rRNA gene sequencing.

Design: Genomic DNA was purified from explanted lung tissue of CF patients undergoing transplantation (n=50). Portions of bacterial 16S rRNA genes were amplified from samples using PCR primers that recognize 16S rRNA genes from a wide spectrum of bacteria. PCR products were cloned into a TA vector and on average, approximately 44 insert containing clones were sequenced for each patient sample by automated DNA sequencing. Sequences were compared to 16S rRNA gene sequences in Genbank to identify species of origin.

Results: Sequence analysis from the first 23 patients with over 1000 sequences to date demonstrates a range of complexity of the microflora in CF lungs, some showing a single dominant organism and others showing multiple co-existing species. These include species identified on microbiology (e.g. *P. aeruginosa, B. gladioli*), but also multiple organisms not identified, including several from the *Burkhoderiales* order (e.g. *Naxibacter* sp.) not previously described in the lung. Sequencing of the remaining specimens in ongoing.

Conclusions: The lungs of CF patients harbor a diverse bacterial community. Sequencing of 16S rRNA genes is a powerful method to profile this diversity and to identify organisms of potential significance in this population.

1472 PTK6 Expression in Normal and Neoplastic Lung Tissues

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Background: PTK6 is a nonmyristoylated intracellular tyrosine kinase that is related to members of the Src family. It is expressed in epithelial cells of the skin and GI tract and has been implicated in the regulation of differentiation. PTK6 is also highly expressed in up to two-thirds of primary breast cancers (CA) and breast CA cell lines that have been examined. It has been proposed that PTK6 enhances EGF regulated signaling in breast CA cells. Two published microarray studies have identified PTK6 as a gene that is differentially regulated in lung CA, but PTK6 protein expression and its functions in lung have not been examined. To gain insight about the possible significance of PTK6 in lung CA, we examined its expression in normal and malignant human lung samples.

Design: A set of 52 cases was selected from the archives to include various histologic tumor types as well as normal lung tissues. The set included H+E and unstained sections from 21 squamous cell CA (SqCC) (7 well differentiated (WD) and 14 moderately to poorly differentiated (MPD)), 22 adenoCA (ADC) (9 WD and 13 MPD), 2 large cell undifferentiated CA (LCUD), and 5 areas of normal lung. Unstained sections were stained for PTK6 on a DAKO Autostainer using appropriate controls. Results were evaluated independently by two pathologists (OD and JC). Frequency, intensity and nuclear and cytoplasmic localization were recorded.

Results: Normal bronchial epithelium consistently expressed cytoplasmic PTK6 with moderate intensity. Of the 7 WD SqCCs, 5 showed frequent intense cytoplasmic and nuclear staining while 1 showed focal weak cytoplasmic staining and one was entirely negative. Of the 9 WD ADCs, 3 showed frequent intense staining, 5 showed focal weak staining and one was entirely negative. Of the 14 MPD SqCCs, 8 showed focal weak staining, predominantly cytoplasmic, and 6 were entirely negative. Of the 13 MPD ADCs, 9 showed weak staining and 4 were entirely negative. Of the two LCUDs, one showed focal weak staining and one was entirely negative.

Conclusions: PTK6 is expressed in normal bronchial epithelium and in some malignant primary pulmonary neoplasms. PTK6 is more frequently and intensely expressed in better differentiated tumors particular those of squamous cell histology. These findings suggest that PTK6 may play a role in the differentiation of pulmonary tissues as described in other systems and further study of its role in neoplastic progression is warranted.

1473 Comparison of FISH, PCR, and IHC Techniques in Assessing EGFR Genetic Alterations and Protein Expression in Lung Adenocarcinoma: Relationship to Clinicopathologic Features

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Background: Epidermal growth factor receptor (EGFR) inhibitors have demonstrated promising effects in some patients with nonsmall-cell lung cancer. The purpose of this study was to investigate the relationship between mutation status, copy number and protein expression of EGFR in lung adenocarcinoma, and the correlation between the genetic status and clinicopathologic features.

Design: Forty-nine adenocarcinomas were tested by PCR for EGFR gene exons 19 and 21 mutations. EGFR gene copy number was investigated by fluorescent in situ hybridization (FISH). Immunohistochemical (IHC) staining for EGFR protein expression was scored from 0-3+ according to the Herceptest criteria. Various histologic parameters were examined, including terminal respiratory unit (TRU) morphology, tumor grade, mitotic count using pHH3, and pleural and lymphovascular invasion.

Results: Five of 49 tumors (10%) had EGFR mutation by PCR. All were deletion mutations. Eighteen (36%) had high EGFR gene copy number by FISH, all of which were polysomies. Fifteen (31%) had EGFR protein expression by IHC, of which 13 (87%) were also positive by FISH. EGFR protein expression and increased gene copy number were seen more frequently in tumors with EGFR mutation (3/5, 60% and 4/5, 80% respectively) than in tumors without EGFR mutation (12/44, 27%, and 14/44, 32% respectively). All tumors with mutation were moderately differentiated. TRU histology was identified in 19 cases, including 5/5 (100%) cases with mutation compared to 14/44 (31%) without mutation. All patients with mutation were females versus (24/44) 54% of patients without mutation. 40% of patients with mutation were never-smokers versus only 10% of patients without mutation. The average number of mitoses /10HPF was 37 in mutation positive cases versus 18 in mutation negative cases, 34 in FISH positive cases versus 12 in FISH negative cases, and 29 in IHC positive cases versus 16 in IHC negative cases. There was no difference in age, stage, size of the tumor, or presence of lymphovascular or pleural invasion between cases with or without mutation.

Conclusions: EGFR gene copy number by FISH and EGFR protein expression by IHC identified most but not all cases with mutation. Cases with altered EGFR gene or protein are more mitotically active than cases without alteration. TRU morphology is a good predictor of the presence of mutation.

1474 Immunohistochemical Detection of Gene Expression Subsets of Non-Small Cell Lung Carcinoma

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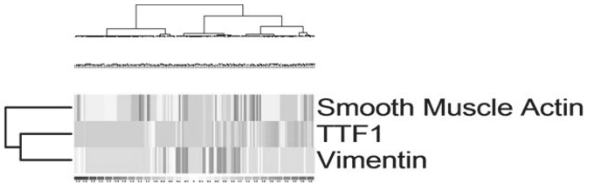
Background: Messenger RNA abundance studies using nucleic acid microarrays allow cluster separation of morphologically similar diseases into molecular subsets. Outcome studies in breast carcinomas and diffuse large cell lymphomas show the relevance of this approach. More recently, lung adenocarcinomas have been subdivided at the mRNA cluster level into magnoid, squamoid, and bronchioid types (Hayes, JCO, in press). The purpose of this project was to pick single representative loci from each cluster, and to screen for distinct clustering at the protein level within an unselected set of non-small cell lung carcinoma (NSCLC).

Design: Duplicate-core tissue microarrays were manufactured from 187 surgically resected primary NSCLC. Cases were unselected for morphology, stage, demographics, risk factors, or outcomes. Screening loci were selected using a rational approach based on gene expression profiling. Loci had to segregate the 3 reported adenocarcinoma subtypes (bronchioid, magnoid, and squamoid), and had to be commercially available. Smooth muscle actin, vimentin, and TTF-1 were chosen. Paraffin immunostains were scored by one Pathologist for signal strength and carcinoma percent positivity. Sample data were included if cores stained for at least one of the 3 markers in at least 10% of cells in at least one of 2 replicate stains. If neither replicate sample stained for any of the 3 markers, it was excluded.

Results: 152 of 187 samples were evaluable. 16 samples were excluded because the tumor or the core was absent, and 19 were excluded due to <10% cells staining for the marker. The staining patterns of the 152 samples are ordered by hierarchical agglomerative clustering, and show distinct clustering into three groups. The Chi square test p value for this distribution of staining is p<2.2e-16.

Conclusions: We have shown that molecular subsets exist within unselected NSCLC, and that these distinguish distinct clusters similar to those seen with mRNA abundance analyses. Future studies will involve defining correlation between message and protein expression in NSCLC, correlation with recognized morphologic subgroups, and correlation with treatment response and survival.



1475 Malignant Pleural Mesothelioma Presenting as Recurrent or Spontaneous Pneumothorax: A Series of 33 Cases

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Background: Diffuse malignant pleural mesothelioma is an aggressive tumor associated with asbestos exposure in 80% of cases. Most patients initially experience chest pain, shortness of breath, unilateral pleural effusions with or without pleural thickening. Rarely MPM presented with spontaneous pneumothorax.

Design: The purpose of the study is to analyse the clinicopathologic features of the largest series to date of MPM presenting with spontaneous or recurrent pneumothoraces collected from the file of the Mesopath group from 1998 to 2006. Analysis of demographic information was performed and asbestos exposure including occupational information were reviewed when available.

Results: From a series of 2244 cases histologically validated according to the procedure of certification of the Mesopath group, 33 were identified with such clinical presentation. The patients were 24 men and 9 women with an age range of 40 to 87 years and an average age of 68 years. In 22 patients a history of asbestos exposure was identified including three women. Occupational information was not available for 2 patients. Pleural plaques were observed in 3 informative patients. Smoking history was yet available in three patients. Five cases presented with a spontaneous pneumothorax, and recurrent pneumothoraces were identified for the others. The diagnosis was not suspected at surgery and was made by histological examination of the resected specimens. Thirty cases were epithelioid, 1 was desmoplastic, 1 was biphasic and 1 sarcomatoid. Anticalretinin immunostains (nuclear and cytoplasmic) were positive in all cases, EMA in 25 cases and was not performed in 4 cases. Antiglandular or antisarcomatous markers were all negative. Seventeen patients are still alive with disease, including one with a survival longer than 3 years.Twelve died of disease with a mean range of 11 months.

Conclusions: Spontaneous or recurrent pneumothorax in a patient over 40 years with asbestos exposure should raise the suspicion of malignant pleural mesothelioma.

1476 P53 and MDM2 Expression in Malignant Pleural Mesothelioma; a Series of 96 Cases

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Background: Although P53 stabilazation has been reported in 25 to 70% of malignant mesothelioma (MM), p53 mutations and LOH at 17p13 locus are very rare. The lack of gene mutations does not explain the effect of p53 on the natural history of MM. It has been suggested that p53 regulation entails a negative control by a protein partner MDM2, itself a cellular oncoprotein. Furthermore, frequent loss of p14 ARF (an inhibitor of p53 MDM2 interaction) leads to inactivation of p53. Additonally, SV40 Tag complex with P53 and RB disrupt the cell cycle control and antagonise p53-induced apoptosis. We analysed a series of MM cases to determine the expression of P53 and MDM2 to evaluate their relationship with SV40 DNA sequences, asbestos exposure, histological type and survival.

Design: Formalin fixed paraffin embeddes tissue sections from 96 cases were retrieved from the MESOPATH files and were immunostained using monoclonal antibodies against P53 (DakoDO-7) and MDM2. The immunoreactivity was semiquantitatively scored according to staining intensity and distribution (0 none, 1+ weak, 2+ and 3+ moderate, 4+ when >75% of cells showed strong nuclear staining). SV40 DNA sequences and p53 mutations (exons 5,6,7,8) were studied by PCR analysis from paraffin embedded specimens and were sequenced. Occupational histories were evaluated by a group of asbestos epidemiological experts. Statistical analysis was performed with chi-square test and actuarial survival by Kaplan-Meier test.

Results: Over expression of P53 (strong nuclear staining-grade 4) was present in 8/58 cases and MDM2 was positive (grades 1-4) in 27/41 cases and there was no correlation with asbestos exposure, histological type, and SV40 status. p53 mutaions were absent in all 38 cases tested. Median survival was better ($p<0.0299$) in those negative (15 months) than in those positive (9 months) for P53. Median survival was better ($p<0.0496$) in those negative (16 months) than those positive (10 months) for MDM2. These appeared to be independent variables for prognosis ($p<0.05$).

Conclusions: p53 mutations were absent in all cases tested but P53 and MDM2 immunopositivity conferred a poorer prognosis which suggests that other factors in the cell cycle influence the P53 protein stabilisation.

1477 Expression and Prognostic Significance of Aurora Kinases in Pleural Mesothelioma

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Background: Current treatment of pleural mesothelioma has yet to significantly improve prognosis. Therefore, there is a need for prognostic prediction before definitive treatment. Previous microarray analysis have identified the Aurora kinases as predictors of aggressive behaviour, but the results were only partially confirmed by immunohistochemistry in an independent cohort. This finding is of potential clinical interest because several small-molecule inhibitors of Aurora kinases A and B have recently been developed. However, the very limited overlap between prognostic gene lists obtained by different groups emphasizes the need for independent validation. The purpose of our study was to confirm the negative effect on survival of Aurora kinases in a separate patient cohort, and to propose a uniform approach to eventually study the predictive role of immunohistochemistry in this setting.

Design: We retrospectively identified 100 patients with pleural mesothelioma. The tumors were classified according to the 2004 WHO Classification. Immunohistochemical studies for Aurora kinase A and Aurora kinase B were performed. Aurora kinase A was considered positive when cells showed both nuclear and cytoplasmic staining. For Aurora kinase B only nuclear staining was scored as positive. At least 1000 cells where counted. Protein expression was correlated with survival and histologic type.

Results: Strong Aurora kinase A expression (>10%) was found in 38/100 (38%) tumors whereas strong Aurora kinase B positivity (>20%) was observed in 43/98 (43%) cases. For all mesotheliomas, strong Aurora kinase B expression was associated with significantly shorter survival than weaker expression ($p<0.0001$), and a similar trend was also noted for Aurora kinase A (log rank: $p=0.08$; Breslow: $p=0.02$). Moreover, among epithelioid mesotheliomas (n=80), patients who had higher Aurora kinase B positivity had shorter survival ($p<0.0001$).

Conclusions: Our data confirm that Aurora kinases are important prognostic factors in pleural mesotheliomas. Acknowledgement: Fondo de Investigaciones Sanitarias 2006-2119.

1478 Immunohistochemistry Panel To Distinguish Primary from Metastatic Squamous Cell Carcinoma of the Lung

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Background: "Primary versus metastasis" always poses a management dilemma when a pulmonary lesion is identified on surveillance CT scan in a patient with known head and neck squamous cell carcinoma (HNSCC). Currently there are no immunohistochemical stains that help in differentiating the two. Nerve growth factor receptor (p75) and cytokeratin subtypes were recently shown to have differential reactivity between HNSCC and lung squamous cell carcinomas (LSCC). Similarly, recent gene expression profiling showed different genetic profiles of LSCC and HNSCC; CK 19 was identified as one such differentially expressed gene. Our aim was to evaluate if an immunopanel with these stains would help in distinguishing HNSCC from LSCC.

Design: Paraffin blocks of HNSCC (n=57) and LSCC (n=22) were retreived from our pathology files. Tissue microarrays with 1.5 mm cores were constructed. Immunohistochemical stains for CK 5/6, CK 7, CK 20, CK 19, CK 903 (high molecular weight cytokeratin), TTF-1, p16, p75, EGFR, Cyclin D1, and c-kit were performed and recorded as positive if greater than 5% of cells showed immunoreactivity.

Results: Results of the relevant immunohistochemical stains are summarized in Table 1.

Conclusions: Although cytokeratin 19 expression was more common in LSCC, the differential expression of keratin 19 seen in genetic profiling could not be demonstrated as an "all or none" immunostaining difference. Similarly, CK 20 and TTF-1 positivity was seen more commonly in LSCC; whereas p75 staining favored a HNSCC. An immunopanel comprising these stains may aid in distinguishing HNSCC from LSCC.

Table 1. Immunoprofile of HNSCC and LSCC

Site	CK7+	CK20+	CK19+	TTF1+	p16+	p75+	EGFR+
Floor of Mouth (n=12)	5(42)	0(0)	6(50)	0(0)	2(17)	12(100)	12(100)
Tongue (n=11)	6(55)	0(0)	7(64)	0(0)	4(36)	8(73)	8(73)
Tonsil (n=10)	1(0)	0(0)	10(100)	1(10)	6(60)	8(80)	8(80)
Pharynx (n=5)	0(0)	0(0)	4(80)	0(0)	2(40)	5(100)	4(80)
Larynx (n=11)	2(18)	0(0)	6(55)	0(0)	3(27)	11(100)	7(64)
Esophagus (n=8)	1(13)	0(0)	5(63)	0(0)	3(38)	7(88)	8(100)
TOTAL HEAD AND NECK (n=57)	15(26)	0(0)	38(67)	1(2)	20(35)	54(95)	47(82)
Lung (n=22)	11(50)	4(18)	22(100)	6(27)	5(23)	16(73)	16(73)

Percent positive in parentheses

1479 Expression of Galectin-3 in 39 Malignant Mesotheliomas (MM): Potential Target for Therapy

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Background: Galectin-3 is a multifunctional oncogenic protein involved in cell adhesion, proliferation, differentiation, angiogenesis and apoptosis. The role of galectin-3 expression has been investigated in carcinomas of the thyroid, gastrointestinal tract, kidney, prostate, liver, breast, and central nervous system where it is believed to play a role in cancer progression. Targeting of galectin-3 has been proposed as a method to improve the efficacy of anticancer chemotherapy. MM is a fatal cancer of the serosal lining for which no therapeutic options that produce prolonged disease-free survival exist. One gene expression profile has shown upregulation of galectin-3 in a small sample of MM (Clin Cancer Res, 2003). We investigated galectin-3 expression by immunohistochemistry in a series of MM to further document the potential for galectin-3 as a target to improve response to chemotherapy in this fatal disease.

Design: We utilized a tissue microarray composed of paraffin embedded tissue sections from 39 cases of malignant mesothelioma. The microarray was immunohistochemically stained for galectin-3 (1:250, Vector), and the tissue sections from each case were evaluated for positive staining. The staining was quantified as 1+ (weak), 2+ (moderate), or 3+ (strong). The pattern of staining was described as focal, diffuse, or patchy (used for sections staining with both focal and diffuse characteristics).

Results: Positive staining for galectin-3 was seen in 27 of 39 cases of malignant mesothelioma. Of these positive cases, 56% (15/27) exhibited strong (3+) staining, 33% (9/27) exhibited moderate (2+) staining, and 11% (3/27) exhibited weak (1+) staining. With regards to the pattern of staining, 37% (10/27) stained diffusely, 33% (9/27) stained focally, and 30% (8/27) exhibited patchy staining.

Conclusions: The frequent strong expression of galectin-3 in our series indicates that galectin-3 plays a role in the progression of MM. This finding suggests that galectin-3 may be a target which can be manipulated to improve response to chemotherapy in many MM and that further investigations of this potential are warranted.

1480 Pulmonary-Graft-Versus-Host Disease and the Idiopathic Pneumonia Syndrome Following Bone Marrow Transplantation: Clinicopathological Distinctions

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Background: Pulmonary graft-versus-host disease (P-GVHD) and idiopathic pneumonia syndrome (IPS) complicate bone marrow transplantation (BMT). GVHD is an immunological reaction of donor's lymphoid cells against recipient organs. With IPS, transplant engraftment produces cytokines that aggravate an injured lung damaged by conditioning. Although of distinct pathogenesis, IPS and P-GVHD are confused clinically and pathologically.

Design: To better define their distinguishing clinical features, we reviewed retrospectively 11 patients with P-GVHD and 10 with IPS diagnosed histologically. P-GVHD was defined as a predominantly T-lymphocytic bronchiolitis and interstitial pneumonitis (lymphocytic alveolitis), with or without organizing pneumonia. IPS was defined by an interstitial pneumonitis (cellular and/or fibrosing NSIP) in the absence of an identifiable infectious etiology.

Results: The patients with P-GVHD (1 black and 10 white males; 1 white female) were 51 $\pm$ 8 yrs old (mean $\pm$ SD). All had undergone allogenic BMT for leukemia, lymphoma, or multiple myeloma. The time from BMT to biopsy diagnosis was 11.1 $\pm$ 7.8 mos (earliest 4 mos). All patients had at least 1 other organ (skin, colon, or liver) involved with biopsy-proven GVHD. None had CMV in the serum. All patients were being treated for GVHD or given steroids upon diagnosis of P-GVHD. Six patients died with evidence of disease. Five patients were alive with evidence of disease from 3 to 14 mos follow-up. Patients with IPS (5 males and 5 females; 1 black female) were 50 $\pm$ 11 yrs in age. All but 1 patient (autologous stem cell transplant) received an allogenic transplant for leukemia, lymphoma, or multiple myeloma and had been conditioned with chemotherapy. The time from BMT to diagnosis was 2.3 $\pm$ 0.9 mos. None of the patients had GVHD. Seven of the 8 patients tested had CMV in the serum. Treatment was supportive, including steroids. All 10 patients died of their disease.

Conclusions: As defined here by histological criteria, P-GVHD and IPS are homogeneous and distinct clinical entities. IPS occurs within 3 months of BMT, is

likely to be accompanied by CMV viremia, and is without evidence of GVHD. The diffuse and temporally uniform interstitial pneumonitis is usually fatal. P-GVHD, by contrast, is a later complication of BMT, occurring in the absence of CMV viremia and in association with GVHD elsewhere. The disease responds to steroids and is not inevitably fatal.

1481 The Importance of Pathological Study on Native Lungs Requiring Transplantation: The Padua Experience

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Background: Lung transplantation is a successful therapeutic option for an increasing range of pulmonary conditions in which the diagnosis is often clinical or based on limited biopsy material. Post-transplantation complications and disease recurrence may relate to the primary disease, and accurate diagnosis of removed native lungs is therefore essential.

Design: We performed an extensive pathologic review of 149 native lungs over a 11-year period at the Institute of Pathology (1995-2006), University of Padua, Italy (heart-lung = 3, single lung = 55, double lung = 91). All lungs were adequately sampled (at least 10 blocks per lung). Routine staining included hematoxylin and eosin, van Gieson, Movat's pentachrome and special stains for microorganisms.

Results: Pathological diagnosis was idiopathic pulmonary fibrosis (IPF, 47), cystic fibrosis (CF, 30), emphysema (30), bronchiectasis (17), pulmonary lymphangioleiomyomatosis (LAM, 5), sarcoidosis (3), Langerhans cell histiocytosis (LCH,2), primary hemosiderosis (IPH, 2), hypersensitivity pneumonia (HP,3), desquamative interstitial pneumonia (DIP,2), primary pulmonary hypertension (PPH, 2), non-specific interstitial pneumonia (NSIP,1), adult respiratory distress syndrome (ARDS,1), post-radiation fibrosis (1), obliterative bronchiolitis (1) pulmonary fibrosis in scleroderma (1) and graft-versus-host disease (GVHD, 1). We found 15 significant discrepancies (7 , table 1) or additional features (8) likely to effect outcome. Additional diagnoses included adenocarcinoma (2 in IPF), carcinoid (1 in a patient with bronchiectasis) mycobacteriosis (3 , 2 in IPF and 1 in IPH) and aspergillus pneumonia (2, in bronchiectasis and CF). Discrepancy and additional diagnoses rate were 7 of 149 (4.7%) and 8 of 149 (5.4%) lung transplantation, respectively.

Conclusions: Our data emphasize the importance of extensive pathological study on native lungs in order to obtain: a) precise epidemiology of lung diseases requiring lung transplantation; b) crucial information for a more correct clinical monitoring of transplanted patients.

Discrepancies of referral and explant diagnoses:

Referrral diagnoses	Explant Diagnoses	Transplantation type
IPF	Non specific interstitial pneumonia	Single
IPF	Desquamative interstitial pneumonia	Single
IPF	Desquamative interstitial pneumonia	Single
IPF	HP	Single
IPF	HP	Single
Sarcoidosis	IPF	Double
LCH	Bronchiectasis	Double

1482 Description of a Novel Subtype of GCDFP-15 Positive TTF-1 Negative Pulmonary Adenocarcinomas

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Background: Gross cystic disease fluid protein (GCDFP) is expressed by cell types with apocrine features, including acinar structures in salivary glands, apocrine glands of the axilla, eyelid, vulva and ear canal, and sweat glands of the skin. Certain non-apocrine cells can also express this protein, including serous cells of salivary glands, submucosal glands of bronchi, and accessory lacrimal glands. However, there are no studies demonstrating GCDFP expression in primary lung neoplasms. Traditionally, an immunohistochemical profile showing positive staining for GCDFP and negative staining for thyroid transcription factor-1 (TTF-1) in carcinomas of the lung has been taken as highly suggestive of metastatic carcinoma of breast, or skin appendage.

Design: Histological sections and tissue microarrays were created from archival paraffin embedded tissue samples from 393 patients with lung carcinomas, of which 207 were adenocarcinomas. These sections were stained immunohistochemically with antibodies to TTF-1 and GCDFP-15.

Results: GCDFP-15 staining was present in normal submucosal glands which were negative for TTF-1. 18 tumors stained positive for GCDFP-15, including 12 of 207 adenocarcinomas, 2 of 119 squamous cell carcinomas, 1 of 22 carcinoid tumors, 1 of 9 non-small cell tumors, 1 of 8 poorly differentiated tumors, and 1 of 5 neuroendocrine carcinomas. Of the 12 adenocarcinomas positive for GCDFP-15, 8 were TTF-1 negative ($p=0.001$, Mcnemar test for pairs and O.R.=0.2), demonstrating an inverse correlation between these two markers. Primary breast or skin appendage tumors were ruled out by clinical history. ER and PR stains were negative on all of the GCDFP positive cases.

Conclusions: This study is the first to demonstrate an inverse correlation between TTF-1 negative and GCDFP-15 positive primary lung adenocarcinomas. Although this represents a relatively small proportion of primary lung carcinomas, these findings indicate that a primary lung neoplasm should not be ruled out in tumors with a GCDFP-15 positive/TTF-1 phenotype. GCDFP-15 positive bronchogenic carcinomas may represent a histogenetically and phenotypically distinctive subset of pulmonary tumors originating from submucosal bronchial glands.

1483 GSK3 Blockade Prevents Bleomycin Induced Lung Inflammation and Fibrosis

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Background: Glycogen synthase kinase 3 (GSK3) modulates inflammatory cytokines production. Since bleomycin causes lung injury which is characterized by an inflammatory response followed by a fibrotic degeneration, we postulated that blockade of GSK3 activity by a GSK3 inhibitor could affect the inflammatory and pro-fibrotic cytokine network which sustains bleomycin induced pulmonary inflammation and fibrosis.

Design: To investigate the effect of GSK3 inhibition on lung inflammation and fibrosis upon bleomycin challenge. The effects of GSK3 inhibitor SB216763 was evaluated on a bleomycin-induced lung fibrosis model in mice.

Results: SB216763 prevented lung inflammation and the consequent fibrosis when co-administrated with bleomycin. Bronchoalveolar fluid analysis of mice treated with bleomycin associated with SB216763 revealed a significant reduction of bleomycin induced alveolitis. Furthermore, significantly lower levels of inflammatory cytokines produced either by macrophages or T helper 2 lymphocytes were determined. Bleomycin treated mice randomized to receive SB216763 develop pulmonary fibrosis to a much lesser extent as assessed by histological analysis.

Conclusions: These findings suggest that GSK3 inhibition has a protective effect on lung fibrosis induced by bleomycin and candidate GSK3 as a potential therapeutical target for preventing pulmonary fibrosis.

1484 Osteoponin and Hypoxia-Inducible Factor-1 Expression in the Lungs of 23 Patients with Sickle Cell Hemoglobinopathy

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Background: Pulmonary hypertension (PH) seen in 40% patients with sickle cell disease (SCD) may be related to hemolysis, anemia, and transfusions. Osteopontin (OPN), a multifunctional cytokine and adhesion protein is involved in immunity, repair, cell survival, and vascular nitric oxide (NO) regulation. OPN is expressed in T lymphocytes and macrophages, and upregulated by smooth muscle cells and macrophages during inflammation and injury. OPN also inhibits expression of NO and prostaglandins. Hypoxia inducible Factor (HIF-1), a transcription factor expressed under hypoxic conditions is involved in pathogenesis of hypoxia-induced PH in rats. We investigated HIF-1 and OPN immunoexpression in lungs of SCD patients to explore possible role in pathogenesis.

Design: Paraffin-embedded lung blocks from 23 autopsied adults were immunostained using standard avidin-biotin peroxide method, and HIF-1 (1:2,000; Abcam Dual Envision), and OPN (1:100; Calbiochem Dual Envision) antibodies. Immunostained slides were graded 0= no staining, 1=weak, and 2= strong. Pulmonary endothelium and smooth muscle (SM), airway epithelium, alveolar macrophages (AM) and type II pneumocytes (II PN) were graded. Statistical analysis were performed using Fisher's Exact test and Chi-Square.

Results: Mean age=41 years (14 M=35, 9 F= 51). Hemoglobin S = 23% to 98%; 9 had HgbS <40%, 15 had SCD (HgbS >40%). All lungs showed PH, (plexiform lesions=57%). Strong OPN expression was seen in pulmonary SM (72%), AM (90%), and II PN (81%); moderate expression in endothelium (50%) and bronchial epithelium (59%). Plexiform lesions had strong expression of OPN in all except one case. Strong HIF-1 expression seen in AM (90%), II PN (90%), and moderate in endothelium (60%) and airway (70%). Vascular SM and plexiform lesions had weak expression (15% and 5%) of HIF-1. Males had more severe SCD, and higher incidence of sudden death (43% vs 11%) compared to females ($p=0.004$).

Conclusions: Strong immunoexpression of OPN and HIF-1 in alveolar macrophages and type II pneumocytes in SCD, suggests a possible role of these cells in PH. Vascular smooth muscle with strong expression of OPN, but not HIF-1 may also have a role. Both OPN and HIF-1 may be involved in pathogenesis of PH in SCD, however, further studies are needed for confirmation. OPN may be a target candidate for therapy / prevention of PH in SCD.

1485 Granulomatous Reaction to *Pneumocystis jirovecii*: Clinicopathologic Review of 16 Cases

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Background: Prior studies of *Pneumocystis jirovecii (formerly Pneumocystis carinii)* pneumonia describe granulomas as a rare, atypical histologic finding. However, in our consultation practice, it appears as a more prevalent reaction to this organism. We reviewed 16 cases to better characterize clinical and pathologic features of this infection.

Design: 27 cases diagnosed as granulomatous *Pneumocystis* infection from 1984-2004 were retrieved from our files. Non-pulmonary cases were excluded. Clinical, histomorphologic, and histochemical details in the remaining 16 cases were reviewed.

Results: 16 cases included 13 males and 3 females ranging in age from 30 to 81 years (mean, 52). Presentation was acute (3/16) or chronic (13/16) with initial symptoms of dyspnea (4/12), cough (4/12), fever (2/12), and incidental pulmonary mass (2/12). Patients had impaired immunity, including HIV/AIDS (7/15), lymphomas/leukemias (4/15) and solid tumors (4/15). Patients had immunosuppressive treatments (5/7), history of PCP prophylaxis (1/7), or prior PCP (1/7). Radioloic findings were diffuse (4/12) or nodular (6/12) pulmonary infiltrates, or solitary pulmonary nodule (2/12). In 13 patients with follow-up data, 6 clinically resolved, 2 died of disease, and 5 died of disease and other contributing causes. Histologic examination revealed clusters of GMS-positive (16/16) *Pneumocystis* organisms with characteristic morphology of thin-walled nonbudding cysts with dark staining intracystic foci. Organisms were within

well- (12/16) and poorly- (4/16) formed necrotizing (12/16) and non-necrotizing (4/16) granulomas ranging in size from less than 0.1 to 1.3 cm (mean, 0.4). Granulomas were multiple (14/16) or single (2/16) and characterized by predominance of epithelioid histiocytes and sparse lymphocytic infiltrate (16/16). Giant cells (8/16), eosinophils (6/16), fibrous rim (4/16), and cavitation (1/16) were also seen. Foamy eosinophilic exudate was present centrally within some granulomas (4/16). Intraalveolar foamy exudates containing *Pneumocystis* were seen in only 1 case.
Conclusions: Granulomatous reaction to *Pneumocystis* is not uncommon in patients with HIV/AIDS, hematopoietic and solid malignancies; conventional findings of intraalveolar foamy exudates with *Pneumocystis* organisms are rare. In this setting, identification of thin-walled, nonbudding cysts with intracystic dark staining foci avoids misdiagnosis as *Histoplasmosis*.

1486 Oligo- and Polyclonal B Cell Response to Lung Carcinoma Is Common

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Background: The lymphoid response to lung carcinoma has been previously studied, focusing on the T cell, NK cell, and histiocytic infiltrate. Recent reports suggest a role of humoral immunity in tumor biology and disease outcome. The purpose of the current study was to assess the local B cell response to lung carcinomas and further characterize its immunophenotype and clonality.
Design: Sixty-nine (n=69) consecutive, previously untreated lung tumor cases between 2001 and 2003 were retrieved from the archives of Roswell Park Cancer Institute (RPCI). The tumor histologic type, mitotic rate, presence or absence of tumor necrosis, scar, and germinal centers in the tumor infiltrating lymphocytes (TIL) were assessed. A lymphoid response score (LRS) was semiquantitatively generated on H&E sections: 0-none; 1-few; 2-moderate; 3-marked. The plasma cell presence was similarly recorded. Immunohistochemical staining for CD3, CD20, CD43 and bcl-2, was performed on cases with LRS ≥ 2 and scored in a similar fashion. Fifteen cases from all LRS groups were analyzed for immunoglobulin heavy chain rearrangement (IgHr) by polymerase chain reaction (PCR). For statistical analysis, patients were categorized into two groups: minimal LRS (MLRS) (LRS 0-1) and significant LRS (SLRS) (LRS 2-3).
Results: Sixty-nine tumors (52 adenocarcinomas, 9 squamous cell carcinomas, 7 large cell carcinomas, and 1 small cell carcinoma) from 35 men and 34 women (mean age 66) were analyzed. Thirty percent of the patients (21/69) had lymph node metastases at the time of surgery. Thirty five percent (24/69) had germinal center formation. Thirty percent (9/30) of the cases studied had a score of 2 or greater. B cells co-expressed bcl-2 in all cases studied (15/15) and CD43 in only 6% (2/34). Kappa and lambda showed polytypic plasma cells in all cases. A significant association was found between SLRS and MLRS groups and presence or absence of germinal centers (p-value < 0.0001, Fisher's exact test). No significant associations were found when the two groups were compared by age, gender, nodal status, tumor histology, necrosis or grade, mitotic rate, or tumor scar. 60% of analyzed samples showed polyclonal (45%) or oligoclonal (55%) IgHr by PCR. No monoclonal B cell population was identified.
Conclusions: In the current study, B cell TIL expressing bcl-2 were common in lung carcinoma and were found to be oligoclonal or polyclonal by IgHr PCR. The role of the humoral immune response to lung carcinoma and its potential clinical or therapeutic implications warrants further study.

1487 Pahological Study of Malignant Pleural Mesothelioma Resected with Extrapleural Pneumonectomy

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Background: Patients with malignant pleural mesothelioma (MPM) are rarely diagnosed at their early stages. It is not fully recognized what is the earliest event in the development of MPM and how it progresses. The aim of this study was to elucidate the early microscopic changes of MPMs that were removed surgically and confirmed to be MPM by histological and immunohistochemical examination.
Design: Thirteen cases with MPM who underwent exrapleural pneumonectomy between 1995 and 2006 were investigated. We arbitrarily defined mesothelioma at early stage as tumor whose thickness was less than 5mm. We used a panel of immunohistochemical markers to confirm the diagnosis of mesothelioma.
Results: The age of the patients ranged from 40 to 62 and all were male. Two were with Stage IB, five with Stage II, five with Stage III, and one with Stage IV mesothelioma. Five of these patients were designated at early stage according to our definition. There was no visible nodule in these cases. There was pleural cavity between parietal and visceral pleura and the surface of the lung looked normal, but both pleura were fused focally. Microscopically mesothelioma cells proliferated both on the parietal and visceral pleura and invaded into it. They proliferated in solitary, trabecular, papillary, or solid patterns. Microscopic invasion into the lung and/or diaphragm was observed after evaluation of many blocks even in MPM with clinical stage I. Seeding of the mesothelioma cells on the tract of thoracoscopy was observed in two cases with early stage MPM. Recurrence occurred in three of five early stage MPMs.
Conclusions: Histopathologically, there was neither stage IA nor IB case, invasion being observed even in extremely early stage MPMs in our series. Once MPM develops, mesothelioma cells may exfoliate easily into the pleural effusion and disseminate diffusely onto the parietal and visceral pleura, thereafter proliferating as in situ neoplasm before invasive nodules are formed. The results of our study suggest that MPM disseminates, proliferates and invades adjacent tissue rapidly and that it is not practical to categorize stage I into stage IA or IB.

1488 Morphological Analysis of Preinvasive Bronchioloalveolar Carcinoma Noguchi Type B

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Background: Localized bronchioloalveolar carcinoma (BAC) with foci of collapse of alveolar structure (central sclerosis, CS) and peripheral replacement (lepidic) growth of cancer has proved possessing excellent prognosis (Cancer 1995;75:2844).
Design: Of 249 surgically resected adenocarcinomas in our hospital, 12 BACs with CS of Noguchi's type B were morphologically analyzed for 1) findings and lesion size on high resolution CT, 2) whether alveolar elastotic framework is maintained or not on elastica-van Gieson stained sections, and 3) the difference in proliferative activity of cancer cells between central area adjacent to CS and peripheral area by use of Ki-67 and p27/Kip1 immunostainings.
Results: 1) All cases had CT findings of peripheral ground glass appearance and central consolidation. 2) Alveolar elastotic framework in CS of the 12 BACs analyzed was maintained and the space within the framework was filled with more or less granulation tissue and fibrosis. 3) Proliferative activity of tumor cells was evaluated by determining Ki-67 and p27 labeling indices (LIs). Both LI data on all 12 cases showed that proliferative activity of tumor cells was greater in the peripheral (or marginal) area than in the central area.
Conclusions: In BACs with foci of collapse of alveolar structure (Noguchi type B), the alveolar elastotic framework was not disrupted and preserved from peripheral area to central sclerotic area, representing morphological non-invasiveness of this type of adenocarcinoma. In these BACs with CS, proliferative activity was lower in tumor cells adjacent to CS, indicating regression stage of the tumor in this area.

1489 Centrosome Abnormalities in Non-Small Cell Lung Cancer: Correlations with DNA Aneuploidy and Expression of Cell Cycle Regulatory Proteins

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Background: Cancer cells frequently exhibit numerical or structural alterations of chromosomes, which are thought to be caused by continuous chromosome missegregation during mitosis. Among mechanisms involved in chromosome segregation errors, much attention has recently been given to centrosome abnormalities, because of its prevalent occurrence in human cancer including lung cancer.
Design: We investigated the relationship of centrosome abnormalities with DNA aneuploidy and the expression of several important proteins that are involved in cell cycle regulation and apoptosis in 175 non-small cell lung cancer (NSCLC) patients. Centrosome abnormalities were detected with the use of fluorescent immunostaining for gamma tubulin antibody. DNA flow cytometry was performed to detect aneuploidy. The expression of $p16^{INK4}$, p53, pRb, cyclin D1, cyclin-dependent kinase (CDK2) and survivin was assessed immunohistochemically.
Results: Centrosome abnormalities were noted in 29 % of the tumors. The frequency of DNA aneuploidy was significantly higher in the tumors with centrosome abnormalities than in the tumors with normal centrosome ($P = 0.015$). Centrosome abnormalities were significantly associated with the expression of $p16^{INK4}$ and lack of expression of pRb ($P = 0.006$ and $P = 0.024$, respectively). Centrosome abnormalities were not associated with the expression of either p53, cyclin D1, CDK2, or survivin alone, but, interestingly, they were significantly correlated with specific combination of $p16^{INK4}$-positive / p53-positive, $p16^{INK4}$-positive / CDK2-positive, and $p16^{INK4}$-positive / survivin-positive expression ($P = 0.003$, $P = 0.001$, $P = 0.036$, respectively). Clinically, centrosome abnormalities had no prognostic value for NSCLCs.
Conclusions: These results suggest that centrosome abnormalities may contribute to pulmonary carcinogenesis by increasing chromosome instability and be controlled by the $p16^{INK4}$, pRb, p53, cyclin D1, CDK2, and survivin protein expression.

1490 Secretin Receptors in Human Lung Cancers: Overexpression in Bronchopulmonary Carcinoids and in Peritumoral Lung Tissue

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Background: Gut hormone receptors, like somatostatin receptors, are often over-expressed in human cancers, allowing receptor-targeted scintigraphic tumor imaging and radiotherapy. A novel promising receptor for these applications is the secretin receptor: it was recently identified in several tumors arising from tissues physiologically expressing secretin receptor, i.e. gastrinomas, pancreatic ductal adenocarcinomas, and cholangiocarcinomas (Am J Pathol 2005, 167:959; J Hepatol 2006, e-pub Jul 28). Since the lung has been reported to express secretin receptors, we assessed these receptors in human lung tumors.
Design: Non small cell lung cancers (NSCLC; n=26), small cell lung cancers (SCLC; n=10), lung carcinoids (n=29), peritumoral non-neoplastic lung parenchyma (n=32), and lung tissue distant from tumors (n=14) were tested for secretin receptor protein expression with in vitro receptor autoradiography using ^{125}I-[Tyr^{10}] rat secretin and in selected cases for secretin receptor mRNA with RT-PCR.
Results: Secretin receptor protein was identified in 62% of lung carcinoids in moderate to high density (mean density 2138 dpm/mg tissue), in 12% of NSCLC in low density (mean 796 dpm/mg tissue), but not in SCLC. RT-PCR revealed wild-type and spliced variant receptor mRNAs in tumors found secretin receptor positive with autoradiography, but not in autoradiographically negative tumors. In the non-neoplastic lung, secretin receptors were observed along the alveolar septa in direct vicinity to tumors, predominantly to NSCLC, in 50% of cases (mean density 352 dpm/mg tissue), but never in lung tissues distant from tumors. RT-PCR yielded wild-type and spliced variant receptor transcripts only in the peritumoral lung. The main histologic difference between the secretin receptor positive and the secretin receptor negative peritumoral lung tissue was the presence of a neutrophilic exsudate in the former.

Conclusions: Secretin receptors could represent a molecular marker for lung carcinoids. Biologically, secretin receptors may modulate the endocrine activity of tumor cells, analogous to their function in gastrinomas; furthermore, they may play a role in the acute inflammatory reaction in peritumoral lung tissues, e.g. in edema, in analogy to their role in regulating transcellular water transport in bile ducts and pancreatic ducts. For clinical purposes, secretin receptor-targeting of carcinoids for scintigraphic imaging and receptor-targeted radiotherapy may be developed.

1491 Lack of Desmoglein 2 Is an Indicator of Cancer Progression and a Poor Prognostic Factor in Stage 1 Non-Small Cell Lung Cancer

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Background: Desmoglein is a desmosomal glycoprotein of the cadherin family. Our prior data demonstrated that lack of Desmoglein 3 is a poor prognostic factor in lung cancer. Here, we investigated the clinico-pathological significance of Desmoglein 2 in 14 major cancer types and its prognostic significance in non-small cell lung cancer.

Design: We immunohistochemically examined the expression of Desmoglein 2 using two independent tissue microarrays (TMAs): one containing 300 samples of non-small cell lung cancer (NSCLC) and a second containing 1150 cases from 14 major cancer types. Staining results were the sum of signal distribution (0, 1, or 2) plus intensity (0, 1, 2, or 3). Staining results were compared with clinical data using the chi-square test. Follow-up data was available for 253 patients in the NSCLC TMA. Survival analysis was performed using the Kaplan Meier curve, and the p value was generated using the log-rank test.

Results: Negative immunohistochemical staining with Desmoglein 2 was associated with poor survival in patients with stage 1 non-small cell lung cancer ($p=0.012$). In the multiple cancer TMA, 901 cases were scorable with Desmoglein 2 staining negatively associated with lymphatic invasion ($p=0.023$) in the entire cohort, lymph node metastasis in lung adenocarcinoma ($p=0.003$), pleural invasion in lung squamous cell carcinoma ($p=0.016$), and pathological stage (stage 1-2 vs. 3-4) in uterine body carcinoma ($p=0.007$). Desmoglein 2 expression varies by site and is positive in: colon (91%), urinary bladder (79%), pancreas (63%), uterine body (59%), lung SCC (51 %), thyroid (50%), stomach (49%), lung adenocarcinoma (46%), ovary (46%), bile tract (45%), breast (44 %), kidney (30 %), prostate (29%), and liver (11%).

Conclusions: Desmoglein 2 staining is negatively associated with factors indicating cancer progression as well as prognosis in stage 1 non-small cell lung cancer. Desmoglein 2 is widely expressed in cancer with colon cancer the most commonly positive tumor and liver the least commonly positive tumor.

1492 Tumor-Cell Scattering: A Clue To Link Micropapillary Pattern and Lymph Node Metastasis in Lung pT1 Adenocarcinomas

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Background: Lung adenocarcinomas with a micropapillary pattern (MPP), characterized by papillary structures with epithelial tufts lacking a central fibrovascular core, have been reported to have a worse biological behavior, and that the patients with MPP tend to present with advanced stage including lymph node metastasis. However, little is known about the mechanisms involved in MPP-associated lymph node metastasis, and prognostic significance of MPP is not fully understood. The aim of this study was to shed light on a link between MPP and lymph node metastasis, and also to clarify prognostic significance of MPP by investigating pT1 lung carcinoma cases clinicopathologically.

Design: We analyzed 127 cases of pT1 lung adenocarcinoma classified according to the new WHO classification with reference to the presence of MPP, tumor-cell scattering (TCS) in scar tissue, and lymphatic involvement (LI). TCS was defined as markedly resolved acinar or papillary structures of tumors with small clusters of carcinoma cells lying ahead and invading the stroma within the fibrotic focus. LI was immunohistochemically detected using D2-40 (lymph endothelial marker) antibody. MPP features were confirmed by MUC1 immunostaining.

Results: Of the 127 cases, 80 (63%) were MPP-positive and 47 (37%) were MPP-negative. The MPP-positive group was associated with significantly more frequent lymph node metastasis and showed significantly worse survival compared with the MPP-negative group (5y survival, 68.8% vs 87.2%, respectively). Moreover, TCS in scar was significantly more frequent in the MPP-positive group, and TCS was significantly associated with LI and lymph node metastasis. MPP-positive carcinoma cells showing TCS was well demonstrated with MUC1 immonostaining by their inside-out pattern. Finally, in p-Stage I (with no lymph node metastasis) but not in II or III patients, Cox multivariate regression analysis identified MPP as a significant predictor and independent factor for a shorter overall survival.

Conclusions: TCS may be a link between MPP and lymph node metastasis: carcinoma cells with MPP tend to undergo TCS in scar and invade lymphatics in pT1 adenocarcinomas. Furthermore, MPP is a significant prognostic factor to predict a poor survival in p-Stage I patients.

1493 Cytoplasmic and Nuclear Expressions of Survivin Predict Worse Outcome of Thymic Neoplasms

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Background: To investigate the expression of Survivin in thymic neoplasms, and to evaluate Survivin expression in association with clinicopathological variables and in prediction of patient clinical outcome.

Design: A series of 60 patients with thymic epithelial tumors were reviewed and classified according to World Health Organization (WHO) scheme. Key clinical information including Masaoka's staging, patient survival, local disease recurrence, and treatment modality was obtained. Percentage of cytoplasmic and nuclear expressions of Survivin was recorded. Complete absence of staining was considered negative. Chi-square test was used to examine the relationship between expression of Survivin and clinicopathological variables, and Cox Proportional Hazard Regression was used to evaluate the prognostic significance of Survivin expression.

Results: There were 6 type A, 15 type AB, 8 type B1, 6 type B2, 17 type B3, and 8 type C tumors. Fifty-seven patients had available long follow up records. Six of fifty-seven patients died of the disease. Six patients developed local relapse. Nuclear expression of Survivin was expressed in 27 of 60 cases (45%). Eighty-three percent (5 out of 6) stage IV cases showed nuclear expression of Survivin, compared to 41% (22 out of 54) cases at stages I, II, and III positive for this marker ($p<0.05$). Cytoplasmic expression of Survivin was present in 5 of 60 cases (8%), with 3 at stage IV (50%), and 2 (4%) at stages I, II, and III ($p<0.01$). Cox proportional Hazard Regression showed that cytoplasmic expression of Survivin was significantly associated with tumor recurrence (Hazard Ratio = 11.06, $p = 0.003$).

Conclusions: Nuclear and cytoplasmic expressions of Survivin were significantly associated with higher tumor stages of thymic epithelial tumors, and cytoplasmic expression of Survivin was a significant prognostic factor for tumor recurrence.

1494 Immunoexpression of Ki-67, bcl-2, p53, and Tyrosine Kinase Receptors in Thymic Epithelial Tumors; Their Correlation with Histologic Subtypes in the WHO Classification and Their Prognostic Value

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Background: Thymoma is a rare tumor of thymic epithelial cells. Clinicopathologic features may not be sufficient to predict the progression of these tumors because of their heterogeneous histology and varied biological behaviour. The immunoexpression of oncogenic markers in thymic epithelial tumors has been reported recently, however the results are controversial. The investigation of tyrosine kinase expression is of timely relevance considering its potential use as a chemotherapeutic target. There is little information in the literature regarding the biologic role of anti-tyrosine kinase receptor-based therapy in thymoma.

Design: The aim of this study was to correlate the Ki-67 labelling index (LI), p53, bcl-2 with the new WHO classification and Masaoka stage in thymic epithelial tumors. We also attempted to detect tyrosine kinase receptor (c-KIT, her-2/neu, and EGFR) expression to determine possible therapeutic implication. 40 surgically resected thymic epithelial tumors (4 type A, 7 Type AB, 6 type B1, 11 type B2, 6 type B3, and 6 type C) were immuohistochemically assessed on tissue arrays for these markers.

Results: Ki-67 LI was significantly increased in type C thymoma and statistically significant differences were identified between Ki-67 LI and WHO subtypes ($p<0.001$). Overexpression of p53 protein was observed in type B3 (67%) and C thymoma (83%), and also not infrequently noted in type A and AB thymoma (27%). The overexpression of p53 protein was associated with a higher tumor proliferative activity and proliferative potential in thymic epithelial tumors was associated with histology of WHO classification. Bcl-2 was expressed not only in type C but also in type A. There were significant differences between bcl-2 and subtypes ($p<0.001$). Positive staining for c-KIT was present in type C thymoma, whereas other types showed no cytoplasmic immunoreactivity for c-KIT. Expression of EGFR was only noted in type B1, B2, and B3. Her-2/neu expression was not detected in any of the cases.

Conclusions: this study suggest that Ki-67 LI, bcl-2, p53, c-KIT, and EGFR protein expression may be useful markers in differentiating thymoma subtypes. The present study demonstrated that Type C thymoma uniformly expressed high Ki-67 LI, p53, bcl-2, and c-KIT. Therefore these proteins are more implicated in the development of thymic carcinoma (type C thymoma) than thymoma. On account of the overexpression of c-KIT in type C thymoma, patients would probably benifit from anti-cKIT treatment.

1494.5 Histologic Changes in Thymomas Treated with Neo-Adjuvant Chemoradiotherapy

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Background: The treatment of choice for thymoma is surgical resection and the ability to achieve complete resection directly affects patient outcome. Locally advanced thymomas are treated prior to resection with combination chemotherapy and/or radiation therapy to shrink the tumor mass and to render the tumor more amenable to surgical resection. The affects of this neo-adjuvant therapy on thymoma histology have not been previously described.

Design: We reviewed 16 surgical resection specimens from 15 patients with thymoma treated with neo-adjuvant therapy (2 radiation therapy alone, 13 combined chemoradiotherapy). The patients included 10 females and 5 males with a median age of 45 years. The resections were preformed a median of 2 months after completing therapy (range 1-54 months). All cases were classified by WHO criteria. Histologic parameters including tumor pattern, tumor necrosis, hyalinization, degree of cytologic atypia, and lymphocyte quantity were assessed and compared to pre-treatment biopsies (available in 8 cases).

Results: The tumors were Masaoka Stage I (2 cases), Stage III (8 cases), and Stage IV (6 cases) and were predominantly WHO Type B3 (12 cases), with 1 case each of Types A, AB, B1, and B2. Histologic changes included hyalinization (>=25% of tumor in 6 cases), necrosis (>=25% of tumor in 4 cases), cystic change (5 cases), hemosiderin deposition (5 cases), and calcification (4 cases). 10 cases demonstrated at least moderate cytologic atypia, with 5 cases containing bizarre anaplastic tumor cells not present in the pre-therapy samples. In 4 cases (25%), the WHO type could not be assigned due to the marked post-therapy changes; in the remaining 12 cases the WHO type was the same as reported in the pre-therapy biopsy report, although lymphocytes were reduced post-therapy in 5/8 samples. At the time of latest follow-up (median 27 months) 3/15 patients had died of disease, including one patient in whom over 85% of the post-therapy tumor was necrotic/hyalinized. 4/5 patients with anaplastic tumor cells were alive at latest follow-up.

Conclusions: Significant and often marked histologic changes occur following neo-adjuvant therapy for thymoma, including severe cytologic anaplasia and in some cases precluding accurate histologic classification in the resection specimen. These findings underscore the importance of the pre-therapy biopsy in establishing the histologic thymoma type.

1495 Evaluation of Immnuohistochemical Markers p63, 34BE12, CK7, TTF1 and EGFR in Lymphoepitelioma-Like Carcinoma of the Lung

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Background: Lymphoepithelioma-like carcinoma (LELC) commonly occurs in nasopharynx, but can arise in a variety of sites as parotid, thymus and lung, associated with EBV, predominantly in Asian population. Primary lung LELC is a very rare tumor classified by WHO in the undiffrentiated large cell carcinoma category. LELC of the lung may present as metastatic carcinoma in various lymph node sites and be subject of diagnostic challange. Recent reports have foud that LELC of lung are chemo-radiosensitive and suggested additive role of EGFR inhibition. The aim of this study is to better define primary lung LELC and to assess EGFR protein expression by immunostain.

Design: Ten cases of LELC are examined. Four cases are of primary lung LELC (from 1997-2006) and 6 cases for comparison are from other sites (5 nasopharyngeal carcinomas and 1 parotid gland carcinoma). Patient's age range is 35 to 81 years (mean 57.4). Male to female ratio1:1. Of the primary lung LELC 1 was smoker, 2 were non-smokers and 1 unknown smoking status. Immunohistochemistry staining was performed with p63, 34BE12, EGFR and AE1/AE3 in all cases. CK7 and TTF1 were performed only in the primary lung LELC.

Results: In all ten cases the large carcinoma cells were strongly and diffusely positive for p63 nuclear staining, 34BE12 and AE1/AE3 cytoplasmic staining. EGFR protein was overexpressed in all primary lung LELC, parotid LELC and four out of five nasopharyngeal LELC. Primary lung LELC were negative for CK7 and TTF1.

Conclusions: Distinctive and strong p63 and 34BE12 positive immunostaining and negativity for CK7 and TTF1 support a squamous lineage for primary lung LELC. Primary site of origin for a metastatic LELC to the lymph nodes can be determined only on clinical base, provided that regardless of site of origin, LELC have the same immnostain profile. Strong and consistent EGFR protein overexpression in primary lung LELC suggest the use of EGFR inhibitors as adjuvant treatment of these tumors.

Table 1

Site	Age/Sex/Smoker status	p63	34BE12	AE1/AE3	CK7	TTF1	EGFR
lung	53/F/no	+	+	+	-	-	3+
lung	65/M/unknown	+	+	+	-	-	2+ to 3+
lung	63/F/no	+	+	+	-	-	2+ to 3+
lung	72/F/yes	+	+	+	-	-	3+
parotid	81/M	+	+	+			2+ to 3+
nasopharynx	36/F	+	+	+			2+ to 3+
nasopharynx	48/M	+	+	+			2+ to 3+
nasopharynx	71/M	+	+	+			1+
nasopharynx	50/M	+	+	+			2+
nasopharynx	36/F	+	+	+			2+ to 3+

1496 Tissue Microarray Study of Hepsin Expression in Diffuse Malignant Mesothelioma: Potential Target for Therapy

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Background: Hepsin is a type II transmembrane serine protease originally identified in human liver. Hepsin is believed to play a role in tumor proliferation, progression, and metastasis. Increased expression of hepsin is reported in carcinomas of the prostate, ovary, kidney, breast and liver. Due to its low homology to other known proteases, hepsin has been proposed to be a unique target for pharmacologic therapy or targeted molecular therapy in these solid cancers. Novel neutralizing antibodies have been developed for hepsin. Diffuse malignant mesothelioma (DMM) is a solid tumor with a dismal prognosis for most patients and little progress has been made in the development of therapeutic options. We investigated the expression of hepsin in DMM to determine if hepsin might serve as a potential focus for targeted molecular therapy in these tumors.

Design: Sections from a tissue microarray of 3 punches each of 27 DMM were immunostained with Hepsin Polyclonal Antibody (Cayman Chemical, Ann Arbor, MI, 1:100). The immunoreactivity was visualized using Dual Envision Kit (DAKO) with a DAB chromagen. Immunopositivity was recorded for each punch by two observers and an average score was obtained for each tumor. A positive in any punch was considered a positive finding for the tumor.

Results: 27 tumor samples were obtained from 22 males and 5 females in their sixth to eighth decades. 26 of 27 DMM were immunopositive for hepsin.

Conclusions: Hepsin is expressed in the majority of DMM in this study. This suggests that hepsin may be a potential target for pharmacologic therpay or targeted molecular therapy in DMM.

1497 Over-Expressed Separase Moderates Chromosomal Numeric Abnormality and Inhibits Serum Starving-Induced Apoptosis in an Aneuploid Adenocarcinoma Cell Line of the Lung

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Background: Advanced non-small cell carcinomas of the lung (NSCCL) often exhibit chromosome numeric aberration or aneuploidy. This subset of NSCCL is associated with an aggressive clinical behavior and poor prognosis. The molecular events that cause aneuploidy of tumor cells remain unclear. One of the mechanisms probably involves the proteins that regulate chromosome segregation during mitosis. Separase and securin are amongst the important regulators. Securin normally binds to separase and prevents it from releasing the sister chromosomes during anaphase. Over-expression of securin has been shown in many tumors. Investigation into the role of separase in the tumorigenesis or chromosome abnormalities is limited.

Design: Cell lines that originated from adenocarcinomas of the lung were transfected with a plasmid vector that contains separase or esp1 gene. Changes of chromosome numbers were recorded in the cells transfected with the esp1 gene and cells transfected with the empty vector. Tumor cell proliferation and growth were assessed with the 3H-TdR incorporation method. Tumor cell apoptosis induced by serum starvation was studied using flow cytometric analysis.

Results: The esp1-transfected tumor cells expressed at least 8-10 fold more mRNA of separase quantified by the reverse real-time PCR method. Tumor cells with over-expressed separase demonstrated a significant decrease in total chromosome numbers. The near-diploid tumor cell population showed an increase of 3-5 folds comparing the control group. Over-expression of the separase in the tumor cells also inhibited the tumor cell proliferative activity (PI=0.09% vs 0.18%) with a 2-fold reduction in the S-phase fraction. The tumor cells with over-expressed separase were apparently resistant to the serum-starving induced apoptosis.

Conclusions: Over-expression of separase in the aneuploid tumor cells may partially correct the aneuploidy or chromosome numeric abnormality. Over-expressed separase may also inhibit the proliferative potential of a tumor. These findings may be very valuable in designing the future targeted molecular therapy if confirmed in future studies.

1498 The Value of Muscle Markers Expression in Mesothelial Proliferations

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Background: Differentiating mesothelial proliferations from metastatic carcinoma can be challenging when based solely on morphologic examination of small biospies. In these situations, the application of immunohistochemical staining can make a more definitive diagnosis. A recent study (Am J Surg Pathol 2006;30(4):463-469) have suggested that the muscle marker, h-caldesmon, a cytoskeleton-associated protein present in smooth and non-smooth muscle cells, may be a sensitive and specific marker for epithelioid mesothelioma. In this study, we investigated the expression of different smooth muscle markers and their diagnostic utility in distinguishing malignant mesotheliomas (MM) from adenocarcinomas.

Design: Computer search identified 40 formalin-fixed, paraffin-embedded cases of MM. Diagnosis of MM was confirmed by surgical decortication or pneumonectomy with immunostaining studies and/or electron microscopy. Twenty cases of adenocarcinomas were also included in the study, as control. Cases were immunostained for h-caldesmon, a specific marker for smooth muscle tumors, desmin and muscle specific actin. Intensity was graded from 0-3 with a score 0 for no staining and 3 for maximal intensity. The pattern/distribution of reactivity was recorded as focal (<10%) or diffuse (>10%).

Results: Our patient population consists of 32 males and 8 females with a mean age of 64+/-8 year-old. Histologically, cases were classified as epithelioid MM in 14/40 cases, mixed 21/40 cases and sarcomatoid type 5/28 cases. All our cases were negative for h-caldesmon. Desmin and SMA were positive in 12/40 (30%) and 22/40 (55%) for MM. None of the adenocarcinoma cases stained for muscle markers examined.

Conclusions: In contrast to previous report, h-caldesmon is not expressed in mesothelial cells and does not show any significant diagnostic value in discriminating MM from metastatic adenocarcinoma in surgical specimens. Awareness of expression of some smooth muscle markers such as desmin and SMA in mesothelial proliferation is important to avoid pitfalls when evaluating specimens from patients with a history of tumors expressing immunohistochemical muscle differentiation.

1499 Value of Plasma CMV-PCR and CMV-IHC for Detecting CMV Pneumonitis in Asymptomatic Lung Transplant Recipients

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Background: Cytomegalovirus (CMV) is the most common viral infection in lung transplant patients. Plasma CMV (pCMV) detected by Real Time Polymerase Chain Reaction (PCR) testing was implemented at our medical center in March 2005. To date no studies have compared pCMV-PCR to concurrent CMV Immunohistochemistry (IHC) on transbronchial biopsies (TBBx). Our purpose is to better understand the value of pCMV-PCR in predicting CMV pneumonitis.

Design: We conducted a retrospective analysis of 50 consecutive patients who had concurrent pCMV-PCR testing and CMV-IHC on TBBx over a 13 month period. Bronchoscopies were performed on patients who met two clinical criterion; asymptomatic/surveillance and symptomatic (fever and/or decrease in FEV1). TBBx specimens were submitted in 10% neutral-buffered formalin, processed by pulmonary biopsy protocol, embedded in paraffin, and 4µm sections were submitted for CMV-IHC. Immunohistochemistry was performed using DakoCytomation Monoclonal Mouse Anti-Cytomegalovirus (Clone CCH2 +DDG9, 1:50 Dako, Carpinteria, CA). CMV PCR detected Human CMV DNA from the UL123 gene, Exon 4, using plasma as the source. The Roche MagnaPureCompact instrument extracted and purified the CMV DNA from plasma. Real Time PCR Taqman assays detect amplified DNA through the use of fluorescent molecules via Cepheid Smart Cycler.

Results: One hundred fifty-five TBBxs from 50 patients with concurrent CMV-IHC and pCMV-PCR were compared. Irrespective of clinical symptoms, the sensitivity was 100%, but, had low power. The NPV also 100% suggests that asymptomatic patients do not have CMV pneumonitis (Table1). When comparing clinical symptoms and pCMV-PCR, symptoms were poor discriminators of CMV pneumonitis as demonstrated by the low sensitivity, PPV and NPVs. However, in asymptomatic patients, a negative pCMV-PCR implies no disease with 93.8% specificity (Table2).

Conclusions: In all lung transplant patients, a negative pCMV-PCR had a NPV=100% and specificity=93%. This implies that in asymptomatic patients, a negative pCMV-PCR excludes CMV pneumonitis with high reliability, specificity=93.8%, superior to clinical impressions. We recommend that CMV-IHC should not be performed for asymptomatic patients with negative pCMV-PCR.

Table1. CMV IHC vs PCR

	IHC +	IHC -	Total
pPCR +	1	11	12
pPCR -	0	143	143 (PPV=100%)
Total	1	154 (Spec=93%)	155

Table2. Clinical Symptoms vs pCMV-PCR

	Symptomatic	Asymptomatic	Total
pPCR+	6	6	12
pPCR-	51	92	143
Total	57	98 (Spec=93.8%)	155

1500 Value of Silver Stains on BAL Fluid in Lung Transplant Recipients

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Background: Lung transplantation is a viable treatment for patients with end stage disease. Pneumocystis carinii pneumonia (PCP) is uncommon in lung transplant patients on lifelong prophylaxis. During bronchoscopy, either for clinical symptoms or routine surveillance protocols, transbronchial lung biopsies (TBBx) and bronchoalveolar lavages (BAL) are obtained and sent, in accordance with the Lung Rejection Study Group, for H&E and Papanicalou staining, respectively. Many transplant institutions stain TBBx and BAL fluid with Gomori Methenamine Silver (GMS) to exclude PCP. In effort to better understand the value of GMS stained BAL fluid, we conducted a retrospective analysis of patients who had concurrent BALs and TBBxs stained with GMS for PCP.

Design: Fifty consecutive patients on PCP prophylaxis with GMS stained TBBxs and concurrent BALs, between 1996 and 2006, were identified. Bronchoscopies were performed on both asymptomatic/surveillance patients and symptomatic (fever and/or decrease in FEV1) patients. TBBxs were fixed in 10% buffered formalin, processed by pulmonary biopsy protocol, embedded in paraffin, and 4µm sections were stained with H&E and GMS. BAL specimens were treated with Cytolyt, centrifuged 5 minutes, resuspended and processed in a Wescor Cytopro for 3 minutes. The slides were then stained with GMS.

Results: We compared 393 GMS stained TBBxs with concurrent GMS stained BALs, from 50 consecutive patients. There were 283 biopsies from asymptomatic patients and 110 from symptomatic patients. None of the GMS stained TBBxs or BALs were pneumocystis positive. The negative predictive value (NPV) and specificity were 100% (Table1).

Conclusions: None of the 283 asymptomatic and 110 symptomatic patients' biopsies were pneumocystis positive, either on BAL or TBBx (gold standard). In all lung transplant patients on PCP prophylaxis, there were no positives by either GMS stained BAL or TBBX. This suggests that in patients on PCP prophylaxis, routine GMS staining on BAL and TBBx may not be necessary. Despite no positive tests in even the symptomatic patients, clinicians remain obligated to exclude infectious etiologies. While limited by the lack of PCP infected patients in our study, it is likely that in certain clinical scenarios GMS staining is indicated. However, based on our results, the risk of PCP under these clinical circumstances appears to be very low.

GMS Stained TBBx vs BAL

	PCP BAL +	PCP BAL -	Total
PCP TBBx +	0	0	0
PCP TBBx -	0	393	393 (NPV=100%)
Total	0	393	393

1501 Clinicopathologic Study of Type 4 Congenital Pulmonary Airway Malformation (CPAM): Evidence for Distal Acinar Origin

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Background: CPAMs are a group of cystic developmental malformations of the lung that are classified based on the putative anatomic region of origin. Type 4 CPAM was first described in 1994 and subsequently there are only anecdotal reports. Recently there has been increased concern that some of these lesions may represent cystic pleuropulmonary blastoma (PPB). We describe the largest series of type 4 CPAM with detailed clinicopathologic analysis.

Design: All cases of congenital/developmental cystic lesions of the lung accessioned to our consultation service were retrieved and cases of type 4 CPAM identified. Additional cases sent in consultation to one of us were also included. Clinical histories, surgical descriptions and histology slides were evaluated. Lesions were evaluated for cyst lining epithelium, subepithelial stromal components, presence or absence of cambium layer, and TTF-1, p63 and desmin immunostaining.

Results: Among 27 cases, 78% presented in the first six months of life (range prenatal to 4 years), without gender predilection. Respiratory distress and/or tachypnea were the most frequent symptoms (59%). The middle/lower lobes were more often affected (53%), with multiple lobes being involved in 20%. The mean size was 7.2 cm (range 1.7 to 12.5 cm). The cysts predominantly contained air, suggesting a connection with the tracheobronchial tree. Cyst lining epithelium was of alveolar type. The underlying stroma was variably cellular, with lesions from older children showing a more collagenous stroma. Lymphocytes, siderophages, foamy histiocytes and prominent thick-walled vessels were common. Foci of cartilage were seen in eight (29%) cases. Lining cells were keratin and TTF-1 positive and p63 negative. Desmin immunostain was negative for rhabdomyoblasts in all cases.

Conclusions: Type 4 CPAM is predominantly a disease of infancy. The histologic features and immunohistochemistry favor a distal acinar origin with cysts being lined by alveolar-type cells. The presence of thick-walled vessels and focal cartilage supports a hamartomatous origin. Lack of desmin staining in subepithelial small round cells may help prevent a misdiagnosis of cystic PPB.

1502 Differences of Fibroblastic Foci of UIP and Intraalveolar Buds of COP/BOOP, as Measured by Cellular Markers and Growth Factors

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Background: Fibroblastic foci and profusion of fibroblastic foci contribute to irreversible fibrosis in UIP and are correlated with increased mortality. On the other hand, the polypoid granulation tissue plug (intraalveolar bud or Masson's body) is one of the pathologic characteristics in COP/BOOP but is not related to progressive interstitial fibrosis. We compared the spatial and quantitative expression of various cell markers and growth factors in UIP and COP/BOOP.

Design: Immunostaining for Transforming growth factor (TGF) beta-1, connective tissue growth factor (CTGF), alpha-SMA, CD34, triptase, S-100, beta-catenin, pSmad 2/3, vascular endothelial growth factor (VEGF), fms-related tyrosine kinase 1 (Flt-1), kinase insert domain region containing receptor (KDR) / Fetal liver kinase (Flk1) and proliferation cell nuclear antigen (PCNA) was carried out in paraffin embedded sections of lung from 12 video-assisted thoracosurgery (VATS) biopsies with UIP and from 10 VATS biopsies with COP/BOOP using a standard indirect avidin-biotin horseradish peroxidase method.

Results: Myofibroblastic proliferation was greater in the fibroblastic foci in UIP than in the intraalveolar buds in COP/BOOP. Capillary proliferation is frequent in intraalveolar buds but scarce in fibroblastic foci. TGF beta-1 and CTGF were expressed strongly in fibroblastic foci but faintly in intraalveolar buds. TGF beta-1, beta-catenin, pSmad 2/3, VEGF, Flt-1 and Flk-1 were seldom observed in pneumocytes adjacent to the lesions in COP/BOOP but were frequently observed in regenerating type 2 pneumocytes and bronchiolar epithelial cells in UIP. PCNA-positive pneumocytes, bronchiolar epithelial cells and myofibroblasts were frequent in UIP not in COP/BOOP.

Conclusions: Differences in expression of growth factors in myofibroblasts and regenerating pneumocytes in UIP when compared to COP/BOOP may explain the difference in the natural history of the two diseases.

1503 Novel Benign Pulmonary and Chest Wall Lesions as Part of the Von Hippel Lindau Syndrome Mimicking Metastatic Renal Cell Carcinoma

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Background: VHL is an autosomal dominant cancer syndrome in which affected individuals and kindreds are at risk to develop renal cell carcinomas (RCC), and other tumors. Distinct lung/chest wall lesions have not been previously reported in patients with this syndrome.

Design: Ten patients, members of VHL families, were evaluated for renal and or other tumors as part of clinical screening. Patients ranged in age from 15 to 59 years. Four were female and 6 were male. Clinical symptoms included chest pain, presence of a chest wall mass and in four patients the lesions were an incidental finding. Five patients had RCC and one a pancreatic neuroendocrine tumor. One patient had been diagnosed as Malignant Mesothelioma.

Results: Eight patients had lung lesions, one a pleural mass, and another one a chest wall tumor. Six patients underwent surgical procedures: thoracotomy in 5 and excision of chest wall mass in 1. Four patients are being followed clinically for benign lung cysts. The clinicoradiological impression was metastatic RCC in 4 patients and malignant mesothelioma in the remainder patient. Morphologically, three of the lung lesions consisted of multiple, variable sized cysts lined by cuboidal cells with clear cytoplasm of uncertain histogenesis. They were distributed within the lung parenchyma, near bronchial structures or in pleural/subpleural locations. The fourth lung lesion consisted of an intraparenchymal proliferation of tubuloglandular structures, lined by clear cells and surrounded by dense myofibroblastic stroma. The chest wall lesion consisted of a small tumor mass characterized by clear cells arranged in tubuloglandular structures with myoepithelial proliferation in the walls. The pleural tumor consisted of a proliferation of papillary structures lined by clear cells. Immunohistochemistry was performed for TTF1, calretinin, CK, SMA, CEA, neuroendocrine and other markers markers. All patients are alive 1 to 7 years after diagnosis.

Conclusions: We describe novel lung and chest wall lesions and propose their inclusion in the spectrum of tumors associated with VHL syndrome. Lung lesions can be cystic or solid. Chest wall tumors are solid and frequently misdiagnosed as metastatic RCC. Recognition of these new lesions as part of the VHL syndrome is important to avoid unnecessary surgery and wrong forms of treatment.

1504 Convergence of College of American Pathologists (CAP) Protocol Model and North American Association of Central Cancer Registry (NAACCR) Elements for the Development and Deployment of Common Data Elements: An Emerging Standard for Mesothelioma Virtual Biorepository

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Background: The rise of molecular and systems biology in medicine is driving development of well annotated and properly characterized bio-repositories to provide tissue to support translational research. Clinical annotation of tissue samples – is central to the success of these repositories as such annotation allows samples can be better matched to the research question at hand and experimental results better understood and verified. To facilitate and standardize clinical annotation in bio-repositories, we have

combined two accepted and complementary sets of data standards, the CAP (pathology data) and NAACCR elements (epidemiology, therapy and progression). Combining these approaches one can create a set of ISO-compliant common data elements (CDE) for oncology tissue banking.
Design: The purpose of the project is to develop a core set of annotation data elements for mesothelioma based on the elements from CAP protocol and the NAACCR checklist. We have associated these elements using modeling architecture to enhance both syntactic and semantic interoperability. The system has a Oracle based three- tiered architecture. The application uses the http server to generate dynamic pages from the database to the users.
Results: We have developed the CDEs for the tissue banks using controlled vocabulary, ontology and semantic modeling methodology. The CDEs included for each case are of different types that include demographic & epidemiologic data, clinical history, pathology data including block level annotation, and outcome data including treatment, recurrence and vital status.
Conclusions: The CAP and the NAACCR elements represent widely established data elements that are used in many cancer centers. Herein we have shown that these representations can be combined and formalized to create a core set of annotation for banked mesothelioma specimens. Because these data elements are collected as part of the normal workflow of a medical center, data sets developed on the basis of these elements can be easily implemented and maintained.

1505 Molecular Profiling of Large Cell Neuroendocrine Carcinoma of Lung and Evaluation of ASCL-1 and KLK11

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Background: Large cell neuroendocrine carcinoma of lung (LCNEC) is a diagnostic challenge, because of its morphological spectrum. Although there are published morphologic criteria and several useful immunohistochemical markers, these lack specificity. More specific and sensitive biomarkers are needed for accurate diagnosis and to better define biological features of neuroendocrine tumors.
Design: 108 tumor specimens (7 LCNEC,100 adenocarcinomas (AD) and 1 small cell carcinoma (SCLC)) were examined for gene expression profile using Affymetrix U133 genechip. Diagnosis was established by morphology and immunohistochemistry (IHC) for synaptophysin (syn), chromogranin A (chr) and CD56. Expression of mRNA was analyzed by t test, and two candidate genes (achaete scute complex, drosophila, homolog of, 1, ASCL1, and kallikrein 11, KLK11) were selected as neuroendocrine markers, and examined by quantitative realtime PCR (qRT-PCR), using RNA from frozen and formalin-fixed paraffin-embedded (FFPE) samples.
Results: 154 probe sets were differentially expressed between LCNEC and AD ($p<0.005$ and > 3-times fold change). ASCL1 and KLK11 were selected for further examination. qRT-PCR of RNA from frozen samples showed ASCL1 expression of LCNEC (mean of relative ratio to TBP = 438) and SCLC (1052) was higher than that of AD (49) and NL (9) ($p<0.001$). KLK11 expression was also higher in LCNEC (6.1) than SCLC (0.2), AD (3.2) and NL (0.9) ($p<0.001$). Using FFPE specimen, reliable results of qRT-PCR were obtained in 27 out of 38 samples (7 LCNEC and 20 AD). RNA quality was inadequate for the remaining 11. The ASCL1 expression was higher in LCNEC (13901) than AD (176) ($p=0.015$), although that of KLK11 did not show significant difference (LCNEC 2.0 vs. AD 2.1). ASCL1 expression ($p=0.001$) and immunoreactivity of chr ($p=0.002$) showed association with histology, but KLK11 expression, immunoreactivity of syn and CD56 did not. ASCL1 expression associated with immunoreactivity of syn ($p=0.002$) and chr ($p=0.003$), but not with CD56. The sensitivity of qRT-PCR of ASCL1 in LCNEC (LCNEC 100%) was higher than immunohistochemical reactivity (syn 83%, chr 83%, CD56 67%).
Conclusions: ASCL1 is a potential useful marker for neuroendocrine differentiation of lung cancer (both of LCNEC and SCLC). The qRT-PCR for detecting gene expression can be applied to FFPE samples as well as fresh frozen samples if the RNA quality is good. KLK11 seems to be a less specific marker and needs further evaluation.

1506 Necrotizing Granulomas Negative for Microorganisms: Clinical Course of 50 Cases

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Background: No organisms are identified using histochemical techniques in a subset of necrotizing granulomas with histologic features suggestive of infection. Some of these are granulomatous infections in which organisms are undetected by light microscopy while others may represent non-infectious disorders. This study was performed to determine the clinical course of patients with unexplained necrotizing granulomatous inflammation.
Design: We retrospectively reviewed 50 cases of necrotizing granulomas in surgical lung specimens that were negative for microorganisms using Grocott Methenamine Silver and Auramine-Rhodamine stains. Only patients with radiologically solitary or multiple nodules were included. Microbiological, clinical and radiographic data were reviewed till the date of last follow-up.
Results: 28 women and 22 men had a mean age of 57 (range 10-82 years). Radiographically, nodules were solitary in 24 and multiple in 26. The nodules were mainly excised by wedge resection (88%). Cultures of the tissue specimens were positive in 9 cases: M. avium-intracellulare complex (8) and M. tuberculosis (1). M. avium-intracellulare complex was isolated from induced sputum in one additional patient. Following lung biopsy, clinical diagnoses were established in 22 patients: granulomatous infection (13), sarcoidosis (4), rheumatoid nodule (2), limited Wegener's granulomatosis (1), pulmonary vasculitis of indeterminate etiology (1) and ANCA-negative necrotizing granulomatous vasculitis (1). Follow-up was available in 45 patients and ranged from 1-134 months (mean, 34 months). Only 10 patients received therapy following resection, including Itraconazole (3), anti-tubercular therapy (3), immunosuppressants (2), Infliximab (1) and trimethoprim-sulfamethoxazole (1). There was no recurrence of nodules in 43 patients. Two patients developed an additional pulmonary nodule, but these remained stable on follow-up.
Conclusions: More than half of patients with necrotizing granulomas in surgical lung biopsies that are negative for microorganisms on special stains have unexplained disease. Infections account for the largest group of patients with specific diagnoses. M. avium-intracellulare, in particular, may be more common than previously recognized. In patients without specific etiologies the nodules do not recur and can likely be managed effectively without therapy.

1507 The Diagnostic Accuracy of Fine Needle Aspiration Cytology Versus Core Needle Biopsy for Peripheral Lung Lesions: A Comparative Study

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Background: Fine needle aspiration biopsy (FNAC) of the lung has long been recognized as a useful diagnostic procedure, providing a rapid, accurate and cost-effective evaluation of pulmonary masses. However, there appears to be a growing movement in favor of core needle biopsy (CNB) over FNAC in detecting carcinoma in some organs such as the breast. In this study, we compared the sensitivity and specificity of these two methods in patients with lung masses.
Design: A computer search identified 76 patients with peripheral lung lesions, subjected to CT-guided CNB including 25 cases having concomitant FNAC, in a tertiary academic medical center between January 2003 and August 2006, and compared to a consecutive 100 FNAC of lung masses. FNAC was performed with 20-gauge spinal needle and CNB with 18-gauge needle. CNB samples were initially submitted for touch preparation to determine adequacy. All patients had follow-up histologic confirmation.
Results: In CNB group, 69/76 patients had malignant diagnosis (47 primary lung, 14 metastatic carcinoma, 2 mesothelioma, 2 melanoma, 2 lymphoma, 1 solitary fibrous tumor and 1 synovial sarcoma), 6/76 cases benign (4 granulomas, 2 reactive) and 1/76 atypical. Immunohistochemistry (IHC) was performed in 43 cases that helped in determining the origin of the tumor in 34/76 (45%) cases. The main indication for core biopsy was to perform IHC to identify the origin of the tumor based on past history of another primary or as a result of unusual presentation of primary tumor as multiple lesions at the time of the procedure. Ten and eleven patients developed postprocedure pneumothorax in CNB and FNAC groups, respectively. For FNAC group, 74/100 cases were positive, 20/100 negative, 4/100 cases false negative and 2/100 cases false positive. FNAC sensitivity was 95%, 91% specificity, 74% accuracy, positive predictive value (PPV) 97% and negative predictive value (NPV) 83%, while in core needle breast biopsy sensitivity was 100%, 86% specificity, 92% accuracy, PPV 99% and NPV 100%. The diagnostic accuracy of CNB was higher than the FNAC, which was statistically significant ($p<0.05$).
Conclusions: In our experience, both FNAC and CNB of the lung have similar sensitivity and specificity, although FNAC has slightly less accuracy. CNB contributed to a more definitive diagnosis in 45% of cases with the application of ancillary IHC studies.

1508 A Simple Inflation Method for Frozen Diagnosis of Lung Tissue

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Background: Evaluation of lung tissue by frozen section has often posed difficulties for the pathologist as uninflated lung tissue showed severe artificial atelectasis and frozen artifact. Recently, the demand for intraoperative pathology consultation for minute lung lesions including GGO (ground-glass opacity) has been increasing. Tissue inflation using fixative has been very helpful in the diagnosis of non-neoplastic lung lesions and minute cancerous lesions; however, this method has not been widely employed for frozen section diagnosis of lung tissue. To obviate this problem, we have inflated lung tissue with the embedding medium (OCT compound) for frozen section diagnosis.
Design: To evaluate the effect of embedding medium injection, we prepared serial dilutions of embedding medium with saline and compared the quality of the frozen sections after injection. Normal lung tissue was divided into five groups - no inflation, inflation with saline, and inflation with diluted embedding medium (1:1, 2:1, 2:3) - and processed for frozen section.
Results: On the basis of morphological assessment, inflating lung tissue by embedding medium diluted at a ratio of 2:3 yielded excellent frozen section quality. Frozen section after inflating saline made lung tissue ragged and difficult to cut on cryostat.
Conclusions: Inflation of lung tissue by embedding medium is a very simple and excellent method for frozen section diagnosis. The minute cancerous and non-neoplastic lesions could be detected more easily by this technique than by the usual method of non-inflated frozen section of specimen.

1509 Pulmonary Veno-Occlusive Changes: An Under-Recognized Finding in Advanced Pulmonary Langerhans Cell Histiocytosis

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Background: Pulmonary Langerhans cell histiocytosis is characterized in its early stages by multiple bronchiolocentric nodules composed of Langerhans cell histiocytes and eosinophils. The majority of patients undergo spontaneous remission or remission following smoking cessation; however, some patients progress to a chronic disease that is characterized by airspace enlargement with cyst formation and alveolar septal fibrosis. These patients show severe pulmonary hypertension. While the cystic nature of the chronic disease is emphasized in the pathology literature, the source of the nearly ubiquitous hypertension has not been as well-characterized.
Design: A search was performed of the surgical pathology and consultation files for cases of advanced Langerhans cell histiocytosis. The cases were reviewed and examined for histologic features including arterial intimal fibrosis and medial thickening, venous intimal fibrosis and muscularization, mineral encrustation of elastic tissue with giant

cell reaction, hemosiderosis, alveolar septal fibrosis with airspace enlargement, and presence of interstitial inflammatory infiltrates. Correlation with radiologic findings on computed tomography and clinical history was performed.
Results: Six cases of advanced pulmonary Langerhans cell histiocytosis were identified. All patients were past or current smokers and showed clinical pulmonary hypertension. All cases showed severe cystic disease by computed tomography. Four cases showed fibrous obliteration of pulmonary veins, one of which also contained focal myointimal thickening of pulmonary arteries. A fifth case revealed only pulmonary arterial thickening without venous changes. The sixth case showed no significant vascular changes. The venous changes were often found in basal regions which lacked significant fibrosis and airspace enlargement. Three cases with veno-occlusive changes also showed associated findings of pulmonary hemosiderosis and mineral encrustation of elastica with giant cell reaction.
Conclusions: In patients with advanced pulmonary Langerhans cell histiocytosis, histologic examination of the lungs frequently shows changes of pulmonary veno-occlusive disease including direct changes of the vessels such as muscularization of venules with intimal fibrosis, and associated changes including pulmonary hemosiderosis and encrustation of elastic tissue with giant cell reaction. These changes may be the cause of the severe pulmonary hypertension which is observed clinically in these patients.

1510 Diagnostic Utility of Thymic Epithelial Markers CD205 (DEC205) and Foxn1 in Thymic Epithelial Neoplasms

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Background: Linage/organ-specific markers are commonly used in diagnostic pathology, e.g., thyroglobulin for thyroid gland; thyroid transcription factor-1 (TTF-1) for thyroid gland and lung. In the thymus, CD205 is linked to "positive selection" process for thymic lymphocytes that take place in the thymic cortex, and Foxn1 is a transcription factor related to thymic organogenesis. Foxn1 also functions to control the expression of certain hair keratins. Given that no thymic organ-specific marker has been described in diagnostic pathology, the potential utility of these antibodies was investigated on thymic epithelial neoplasms.
Design: A total of 73 cases comprised of 58 cases of thymoma (8 cases of type A; 20 of AB; 8 of B1; 16 of B2; and 6 of B3), and 15 cases of thymic carcinoma were retrieved. Immunostains for Foxn1, CD205, CD5 and CD117 were performed. Foxn1 and CD205 were also examined on 43 cases of primary non-small cell lung carcinoma, 71 cases of cutaneous squamous cell carcinoma and 29 cases of cutaneous basal cell carcinoma, and normal tissue from 23 organs/sites.
Results: There were 40 male and 33 female (M:F = 1.2:1), with ages ranging from 23 to 90 years (median, 58 years). Foxn1 was diffusely expressed in all cases of type B thymoma in nuclear staining, whereas the expression was patchy in type A thymoma (96%) and focal in thymic carcinoma (77%). CD205 cytoplasmic expression was strong and diffuse in type B thymoma (100%), strong but focal in type A thymoma (92%), focal with variable intensity in thymic carcinoma (57%). 13% of skin squamous cell carcinomas stained focally for Foxn1. All skin basal cell carcinomas were completely negative for Foxn1. The normal thymus expressed Foxn1 in the epithelial cells, but Foxn1 was completely negative in all other organs, including skin hair follicles. CD205 was expressed in 7% of poorly differentiated non-small cell carcinomas of lung. CD205 was expressed in myeloid dendritic cells of various organs/tissues as well. CD5 was expressed focally in type A thymoma (15%) and type B3 thymoma (28%), whereas it was strongly expressed in thymic carcinoma (67%). CD117 was stained only in thymic carcinoma (69%) in variable intensity and distribution.
Conclusions: Foxn1 is a sensitive and specific marker for both thymoma and thymic carcinoma. CD205 is a sensitive and specific marker for thymoma but its sensitivity to thymic carcinoma is lower than CD5 and CD117.

1511 Trends in Diagnosis of Bronchioloalveolar Carcinoma at University Medical Centers and Affiliated Community Hospitals Following the Shift in the WHO Criteria

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Background: The 1999 WHO classification for Lung Tumors re-defined "Bronchioloalveolar Carcinoma" (BAC) as a type of adenocarcinoma growing along pre-existing alveolar structures without evidence of invasion. For this reason, the definitive diagnosis should be made on resection specimens and not on small biopsy or cytology specimens. The objective of this study was to survey the trend in diagnosis of bronchioloalveolar carcinoma in cytopathology and surgical pathology cases since the change in criteria.
Design: Surgical pathology and cytopathology reports with the term "bronchioloalveolar carcinoma" and "bronchoalveolar carcinoma" in the final diagnosis were searched in our laboratory information system for the years 1999 to 2006. Cases were divided into those diagnosed at the university medical centers and those at the affiliated community hospitals. Within each group, cases were categorized by specific type – cytology, biopsy, or resection. Furthermore, we determined whether each cytology and biopsy case rendered a definitive diagnosis of BAC or a descriptive diagnosis suggestive of BAC. Resection cases were subclassified into those with a definitive diagnosis of BAC and those adenocarcinomas with a bronchioloalveolar component. These cases were correlated with year of diagnosis.
Results: The 4 definitive diagnoses of BAC on small samples at the university medical centers were made in 2000 and 2001 only. In contrast, the 8 definitive diagnoses on small samples at the community hospitals were made continuously from 1999 to 2006. Of the 12 cases definitively diagnosed as BAC on cytology or biopsy, 3 were shown to represent invasive adenocarcinoma on resection specimens.
Conclusions: The absence of definitive BAC diagnosis on cytology and biopsy specimens from the university medical centers after 2001 is a reflection of the specialty oriented practice. Further educational efforts would help reduce the number of BAC diagnoses rendered on small biopsy and cytology specimens, particularly in general practice settings.

BAC Diagnosis at University and Community Hospitals

BAC Diagnosis	Cytology-definitive	Biopsy-definitive	Cytology-descriptive/suggestive	Biopsy-descriptive/suggestive	Resection-definitive	Resection-BA component
University (1999-2002)	1	3	2	6	23	34
University (2003-2006)	0	0	1	3	17	54
Community (1999-2002)	3	1	2	0	12	1
Community (2003-2006)	3	1	0	3	6	10

BAC-bronchioloalveolar carcinoma; BA-bronchioloalveolar

1512 Increased Phosphorylated p70 S6 Kinase (p-p70S6K) Protein Expression in Non Small Cell Lung Cancers

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Background: p70 S6 kinase (p70S6K), an integral member of the mTOR signaling pathway, is a protein kinase that promotes protein synthesis and controls the cell cycle. p70S6K phosphorylation (p-p70S6K) by mTOR incites cell growth and proliferation and has been implicated in tumorigenesis and invasion in several human tumors and cell models. However, the role of p-p70S6K has not been examined in pulmonary neoplasms and the prognostic significance of p-p70S6K expression in non-small cell lung cancers (NSCLC) has not been previously evaluated.
Design: Formalin-fixed, paraffin embedded sections from 116 NSCLC, including 42 squamous cell carcinomas (SCC), 43 adenocarcinomas (AC), and 31 bronchioloalveolar carcinomas (BAC) including both pure BACs and adenocarcinomas with BAC features were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) with polyclonal antibody to p-p70S6K (Thr 421/ Ser 426 antibody, Cell Signaling, Danvers, MA). Nuclear and cytoplasmic immunoreactivity was semiquantitatively assessed in both tumor and adjacent benign lung. Expression was defined as $\geq$ 10% tumor staining. Results were correlated with histologic and prognostic variables.
Results: The adjacent benign lung parenchyma expressed variable nuclear p-p70S6K immunoreactivity in 85% of cases, while minimal cytoplasmic immunoreactivity was noted in only 3% cases. In contrast, cytoplasmic staining was present in 49% tumors, while nuclear staining was variably present in 97% tumors There was a significant difference in cytoplasmic p-p70S6K expression between histologic tumor types, with SCC showing 67% expression in contrast to 33% and 48% in AC and BAC, respectively (p=0.007). Cytoplasmic expression was significantly increased in larger tumors for all histologic subtypes (60% >3.0cm vs. 39%<= 3.0 cm; p=0.033). Interestingly, within ACs, increased cytoplasmic expression correlated with low tumor stage [0% Stage IV, 20% Stage III, 83% Stage II and 26% Stage I, p=0.023)].
Conclusions: Expression of p70S6K appears to differentiate between SCC, AC, and BAC. Greater tumor dimension is associated with increased expression of p70S6K, suggesting that signals for cell growth and survival are enhanced as tumor mass increases. However, advanced tumor stage may be associated with a relative loss of p70S6K expression. These findings that p70S6K expression may be associated with NSCLC tumorigenesis and prognosis warrant further study.

1513 Pathologic and Molecular Features of Screening Spiral Computed Tomography (SCT)-Detected Lung Cancers

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Background: Detailed studies on the pathological features of SCT-detected lung cancers and comparison with unscreened tumors are still lacking.
Design: We evaluated the histopathologic features of 69 SCT-detected lung cancers radically resected at the participating Institutions between 2000 and 2006, and collected from about 6,000 high-risk subjects (50 years or more, minimum of 20 pack-year index) undergoing annual low-dose single-slice SCT. Tumors were classified according to the 2004 WHO system and, for adenocarcinoma, by evaluating central scar diameter and invasion size according to Terasaky et al. (Am J Surg Pathol 2003;27:937).
Results: There were 47 males and 22 females (range 50-74 years), with 52 adenocarcinomas (AC), 11 squamous cell carcinomas (SCC), 4 large-cell carcinomas (LCC), and 2 small-cell lung cancer (SCLC), measuring from 0.4 to 6 cm. Overall, 37 (54%) tumors were stage IA (29 AC, 4 SCC, 3 LCC & 1 SCLC), 11 (16%) stage IB (7 AC, 3 SCC & 1 SCLC), 2 (3%) stage IIA (all AC), 5 (7%) stage IIB (3 AC & 2 SCC), 10 (15%) stage IIIA (7 AC, 2 SCC & 1 LCC), 3 (4%) stage IIIB (all AC), and 1 (1%) stage IV (AC). 8 were G1, 25 G2 and 30 G3 (6 post-chemotherapy tumors were not assessed). 29 (56%) adenocarcinomas showed mixed pattern with replacement-type growth by bronchioloalveolar (BAC) component (predominantly of non mucinous type) ranging from 8 to 100% (median 40%), whereas 23 (44%) showed nonreplacement-type growth with acinar or solid features. Central scar diameter and invasion size were smaller in mixed AC (median values 8 and 6 mm) than in other AC types (both 15 mm) (p=0.007 & p<0.001, respectively). As compared with other 79 consecutive unscreened lung carcinomas, CT-screened tumors showed a prevalence of stage IA tumors (p=0.003) a younger population (60 vs 65 years, p<0.001), and were smaller (16 vs 30 mm, p<0.001), better differentiated (p=0.002), less necrotic (p=0.034), and with higher microvessel density (p=0.043) than unscreened ones. These associations were also retained for stage I tumors. Five of 16 screened AC showed K-ras mutations at codon 12, all GGT (glycine) to TGT (cysteine) transversions.
Conclusions: SCT-detected lung cancers share the same features of fully malignant tumors, despite earlier detection and less advanced clinical stage.

1514 p27^{kip1} Loss Correlates with Poor Prognosis in Lung Adenocarcinoma

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Background: p27^{kip1} is a member of the Cip/Kip family of cyclin dependent kinase (CDK) inhibitors that regulate the cell cycle. Although several studies have shown loss of p27 correlates with poor prognosis in non-small cell lung cancer (NSCLC), some have failed to show significance after stratifying for stage and not all studies report survival data according to histologic type.

Design: We immunohistochemically stained a TMA with 232 NSCLC using the DAKO, Clone M7203 antibody to p27^{Kip1}. A score for each case was made based on creating a sum of the distribution and intensity of staining. A score of absent or weak staining was regarded as negative and moderate or marked staining as positive. Follow-up was available in 185 cases. Kaplan Meier and Cox survival analysis and chi-square statistics were made using SPSS 13.0.

Results: p27 was positive in 84 of 232 cases (36.2%) with no significant difference in staining in 46 of 124 adenocarcinomas (54.8%) and 38 of 108 squamous cell carcinomas (45.2%). There was no significant difference in staining according to sex, however in women there were significantly fewer positive squamous cell carcinomas (13%) than adenocarcinomas (44.8%) (p=0.046). Cox survival analysis, stratified for all stages, showed a significantly worse survival for all NSCLC patients whose tumors stained negative for p27 (p=0.042). 5-year survival for all NSCLC patients with Stage 1 and 2 tumors was reduced at 33.9% vs 53.3% with negative versus positive p27 staining (p=0.047). This survival difference was strongest for Stage 1 and 2 adenocarcinoma patients (21.3% vs 66%, p=0.008) and no significant survival correlation was seen in squamous cell carcinoma.

Conclusions: Loss of p27 correlates with poor prognosis in NSCLC. This is best seen in early stage NSCLC and in adenocarcinomas rather than squamous cell carcinomas.

1515 Thyroid Transcription Factor-1 (TTF-1) Expression in Pulmonary Neuroendocrine Carcinomas: Clone-Based Variability

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Background: Thyroid transcription factor-1 (TTF-1) is known to be preferentially expressed in a limited number of normal and neoplastic tissues. As such, it serves as a useful diagnostic tool in establishing the primary site of pulmonary and thyroid carcinomas. It's also been reported to be differentially expressed in pulmonary and gastrointestinal carcinoids. However, since only limited studies address the issue of variable TTF-1 expression related to the use of different clones of commercially available antibodies, we investigated this phenomenon on a set of primary pulmonary neuroendocrine carcinomas (NEC).

Design: Tissue microarray based samples of 176 NEC, including 46 typical carcinoids (TC), 31 atypical carcinoids (AC), 27 large cell neuroendocrine carcinomas (LCNEC), and 72 small cell carcinomas (SCLC) from different patients were studied immunohistochemically (IHC) for TTF-1 expression. Surgical resections and biopsy specimens of 16 intestinal carcinoids were studied for comparison. Two common commercially available antibodies raised against TTF-1, 8G7G3/1 from Dako (Carpinteria, CA) and SPT24 from Novocastra (Newcastle, UK) were applied. Based on the nuclear staining of the tumor cells, the IHC results were recorded as positive or negative.

Results: When studied with 8G7G3/1 clone, pulmonary NECs were positive for TTF-1 in 35% of cases: 7% of TC, 24% of AC, 52% of LCNEC, and 54% of SCLC. When studied with SPT24 clone, pulmonary NECs were positive for TTF-1 in 61% of cases: 48% of TC, 48% of AC, 56% of LCNEC, and 78% of SCLC. No significant difference in TTF-1 expression between the two antibodies was found in normal alveolar lining cells. All 16 intestinal carcinoids were TTF-1 negative with both antibodies.

Conclusions: Depending on the clone of antibody, pulmonary carcinoids show significant (up to six fold) variation in TTF-1 expression. Since the SPT24 clone has greater sensitivity, it may prove to be more diagnostically useful; however, further studies are needed to evaluate the utility of different TTF-1 antibodies in establishing a primary site of a NEC.

1516 The Incidence of Neuroendorine Cell Hyperplasia (NEH) in Pulmonary Neuroendocrine Tumours and Non-Small Cell Carcinomas

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Background: NEH is viewed as a preneoplastic lesion for typical carcinoids with its presence reported in two thirds of peripheral lesions, although little is known of its incidence in relation to other neuroendocrine tumours. We have evaluated the incidence of NEH in the background lung of resected central and peripheral carcinoids, other neuroendocrine tumours and non-small cell carcinomas.

Design: Resected cases of typical carcinoids (TCs, n=46), atypical carcinoids (ACs, n=14), large cell neuroendocrine carcinomas (LCNECs, n=18), small cell carcinomas (SCLCs, n=22), adenocarcinomas (ADENOs, n=26) and squamous cell carcinomas (SQCs, n=18) were retrieved from the archives (1982-2006). All cases with histologic evidence of airway obstruction were excluded. Random blocks of normal lung were stained for CD56 (Vector, Vector CA, 1/100 Dilution) and evaluated for the presence of linear proliferations, cell aggregates (more than four CD56 positive cells), and tumourlets (less than 5mm with basement membrane invasion). Clinical data were retrieved and where available were correlated with NEH incidence.

Results: NEH was detected in 46% of TCs, 36% of ACs, 39% of LCNECs, 9% of SCLCs, 15% of ADENOs and 11% of SQCs. Among the TCs there was an increased incidence of NEH in peripheral (n=13, 62%) versus central (n=33, 43%) tumours. There was no association between NEH and smoking history. Lung function (n=2) and HRCT findings (n=7) in patients with TCs showed no evidence of obstructive lung disease.

Conclusions: Our results indicate that the incidence of NEH is increased in the lungs of patients with both TCs and ACs as well as cases of LCNECs compared to SCLCs, SQCs and ADENOs. Peripheral TCs showed a higher incidence of NEH compared to central TCs. The absence of physiological and HRCT evidence for associated obstructive bronchiolitis may reflect a lower density of NEH in this cohort compared to those with symptomatic diffuse idiopathic neuroendocrine cell hyperplasia.

1517 Bronchiolitis Interstitial Pneumonitis: A Distinctive Disease with Clinical and Pathological Features Intermediate between Bronchiolitis Obliterans Organizing Pneumonia and Usual Interstitial Pneumonitis

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Background: Bronchiolitis obliterans with interstitial pneumonitis was categorized by Liebow in 1975 as one of five forms of interstitial pneumonitis. The entity has received little attention since then except in so far as it has been commingled under the rubric of bronchiolitis obliterans organizing pneumonia (BOOP).

Design: We studied thirty-two patients who had respiratory insufficiency severe enough to prompt open lung biopsies. We evaluated clinical and pathological features and compared them with six other pulmonary diseases that share histologic similarities, namely, BO, BOOP, NSIP, UIP, airway-centered interstitial fibrosis, and idiopathic bronchiolocentric interstitial pneumonia. We also quantitated the changes in ten cases of cystic fibrosis, an unrelated disease with both bronchiolar and interstitial pathology.

Results: The common feature of all our cases was a combination of both prominent bronchiolitis and interstitial inflammation and fibrosis but little or no organizing pneumonia, little or no peribronchiolar fibrosis, and no hyperplasia of bronchus-associated lymphoid tissue. None of the patients had clinical evidence of hypersensitivity pneumonitis. After treatment with corticosteroids, seven patients had improvement in symptoms, pulmonary function tests (PFTs), and radiographic findings, five experienced subjective improvement with unchanged PFTs or chest X-ray, one patient's condition was unchanged, two patients' disease worsened, and disease in five patients worsened and led to death.

Conclusions: The natural history of bronchiolitis interstitial pneumonitis (BIP) is better than UIP and worse than BOOP. Response to corticosteroids is not as common as it is in BOOP. On the other hand, BIP does not progress in most patients on corticosteroids. BIP can be considered before lung biopsy in a patient with a combination of alveolar and interstitial disease on radiographs and either restrictive or both restrictive and obstructive disease physiologically. The pathology of BIP is distinctive.

Table 1. Comparison of histologic features in some bronchiolar and interstitial diseases

	BIP	BO	BOOP	NSIP	Airway-centered interstitial fibrosis	Idiopathic bronchiolocentric interstitial pneumonia	CF
Bronchiolar myxoid fibrous tufts	+/++	+++	++	0	0	0	++
Organizing pneumonia	+/++	0/+	+++	0/+	0	0/+	+++
Interstitial pneumonitis	++	0	0	+++	+/++	+++	+/++
Peribronchial fibrosis	0/+	0/+	0/+	0	+++	+/++	++
Low power pattern							

1518 Molecular Predictors of Brain Metastases in Patients with Non-Small Cell Lung Carcinoma (NSCLC)

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Background: Brain metastases in patients with NSCLC are associated with significant morbidity and mortality. No reliable markers are available to predict the patients who are at greater risk to develop brain metastases. In this study, we evaluated the role of EGFR, VEGF-A, VEFG-C, MGMT and E-cadherin in predicting patients who are at greater risk to develop brain metastases.

Design: We studied 64 patients with NSCLC and available brain and pulmonary surgical pathology specimens, treated with surgery between 1995 and 2005 at the Brigham and Women's Hospital. 26 patients with NSCLC developed brain metastases and 38 who never developed brain metastases in the same period served a matched control group. We examined the clinical-pathologic features of NSCLC in brain and pulmonary pathology specimens that had been characterized for expression of VEGF-A, VEGF-C, EGFR, E-cadherin and MGMT. Correlation analyses of each clinical-pathologic characteristic were run against the individual markers to evaluate and quantify associations.

Results: The median follow up was 6.5 years (range, 2.5-17 years). Patients developed brain metastases after a median time of 0.9 years (range, 0.01-7.5 years) after primary diagnosis of NSCLC. Overexpression of VEGF-C and EGFR in lung carcinomas was strongly associated with development of brain metastases ($p<0.0001$ for both), as was loss of E-cadherin and MGMT expression ($p=0.006$ and $p<0.001$, respectively). There was no correlation between the expression of VEGF-A and the risk of developing brain metastases.

Conclusions: Our results show that overexpression of VEGF-C and EGFR, and loss of E-cadherin and MGMT by tumor cells in primary NSCLC is highly associated with development of brain metastases. This panel might be useful in identifying a subset of patients with NSCLC who has a higher risk for developing brain metastases, and therefore should be treated more aggressively.

1519 The MIB-1/Caspase Labeling Index as a Predictor of Metastasis to the Brain in Patients with Non-Small Cell Lung Carcinoma

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Background: Although the initial staging of non-small cell lung carcinoma (NSCLC) is essential for determining the treatment approach, there is no consensus as whether patients with NSCLC should be screened for distant metastases to the brain. The aim of this study is to identify a subgroup of patients with NSCLC who are at greater risk of developing brain metastases and subsequently may benefit from an earlier diagnosis and a more aggressive treatment.

Design: 64 cases with primary NSCLC (40 adenocarcinomas; 24 squamous cell carcinomas) from the files of the Department of Pathology at the Brigham and Women's Hospital were studied. They consisted of 26 patients (study group) who developed brain metastases and 38 patients with no brain metastasis (by brain imaging or neurologic examination). Slides were reviewed and a representative section from the lung primary is selected and immunostained with antibodies against MIB-1 and caspase. Labeling indices (LI) were generated by dividing the number of positive tumor cells by the total number of tumor cells in the area with the highest positivity. MIB-1/caspase index was generated by dividing the MIB-1 LI by the caspase LI.

Results: The study group consisted of 12 men and 14 women with median age of 57.7 years (range: 41.3-75.3 years). The control group consisted of 19 men and 19 women with median age of 64.9 years (range: 39.9-84.0 years). Brain metastases developed a median time of 0.93 years (range: 0.01-7.45 years) after the lung primary. The median tumor size was 2.1 cm (range: 0.3-9.5cm) in the study group and 2.7 cm (range: 0.6-7.3cm) in the control group. The two groups were similar when compared by age, sex, lung tumor size, AJCC stage, and histologic types. MIB-1 LI was higher in NSCLC who later developed brain metastases (32.5 vs. 16.5, p=0.0004). Caspase LI was lower in NSCLC who later developed metastases to the brain (1.5 vs. 4, $p < 0.0001$). Moreover, a higher MIB-1/caspase index was highly associated with NSCLC metastatic to brain ($p < 0.0001$).

Conclusions: Our results show that MIB-1/caspase index is a strong predictor for the development of brain metastases in patients with NSCLC. A higher MIB-1/caspase index characterizes patients who are at greater risk of developing metastatic NSCLC to the brain. These patients should be monitored closely by brain imaging for early diagnosis and treatment of their brain metastases.

1520 The Role of Desmoglein-3 in the Diagnosis of Squamous Cell Carcinoma of Lung

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Background: Desmoglein-3 (Dsg3) belongs to the cadherin superfamily of Ca^{2+} dependent proteins; it has an important role in cellular adhesion of stratified epithelia. Several microarray based studies, including ours, have highlighted the up-regulated expression of Dsg3 in squamous cell carcinoma (SCC) of the lung. The purpose of this study is to determine the specificity of Dsg3 to SCC and to assess the role of Dsg3 expression in the diagnosis of SCC of lung.

Design: Immunohistochemistry staining targeting Dsg3 was performed on 394 samples from 14 different tumor types across 22 tissue types.

Results: Results are displayed in Tables 1 and 2.

Table 1. Organ Specific Immunohistochemistry

Organ	Tumor type	N	Immunopositives (N)	%
Lung	SCC	64	63	98
Lung	AC	47	1	2
Lung	Large cell ca	36	0	0
Pleura	Mesothelioma	8	0	0
Lymph node	Metastatic SCC	20	19	95
Brain	Astrocytoma	9	0	0
Brain	Oligodendroglioma	9	0	0
Skin	SCC	12	12	100
Skin	Malignant melanoma	10	0	0
Head-Neck	SCC	5	5	100
Head-Neck	Mucoepidermoid ca	4	4	100
Tonsil	SCC	10	10	100
Tongue	SCC	8	8	100
Larynx	SCC	9	9	100
Thymus	Thymoma	10	2	20
Esophagus	SCC	15	15	100
Stomach	AC	10	1	10
Pancreas	AC	10	8	80
Colon	AC	9	5	56
Anus	SCC	4	4	100
Liver	Hepatocellular ca	9	0	0
Bladder	Transitional cell ca	10	3	30
Prostate	AC	9	0	0
Vulva	SCC	9	9	100
Cervix	SCC	9	9	100
Ovary	AC	10	1	10
Testis	Seminoma	10	0	0
Testis	Embryonal ca	2	0	0

AC: Adenocarcinoma; ca: carcinoma

Table 2. Group Specific Immunohistochemistry

A. All cases			
	SCC	Non-SCC	Total
Dsg3 (+)	163	33	196
Dsg3 (-)	2	196	198
Total	165	229	394
Sensitivity, 0.99; specificity, 0.86			
B. All lung cases			
	SCC of lung	Non-SCC of lung	Total
Dsg3 (+)	63	1	64
Dsg3 (-)	1	82	83
Total	64	83	147
Sensitivity, 0.98; specificity, 0.99			

Conclusions: Dsg3 is not specific to SCC of the lung; however, it can be useful as an ancillary tool to determine the histologic subtype of lung cancer. Identification of SCC in lung cancer patients may be necessary with the advent of new therapeutic agents, such as VEGF inhibitors, which may be contraindicated in patients with SCC. Further studies focusing on different histologic types of lung tumors should be conducted to elucidate the potential role of Dsg3 in SCC of lung.

1521 MET in Pleural Mesothelioma: Mutational Screening, Phosphorylation Status, and Sensitivity to MET Inhibition

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Background: Recent independent studies have raised interest in the MET receptor tyrosine kinase as a therapeutic target in malignant mesothelioma. MET is the receptor for hepatocyte growth factor (HGF). MET can be activated by HGF, mutations, or strong overexpression.

Design: We performed a screen for *MET* activating mutations (kinase, juxtamembrane, and semaphorin domains) in 14 cell lines and 55 mesothelioma patient samples. The latter were selected from a total of 99 mesothelioma samples based on their relative overexpression of *MET* by Affymetrix U133A microarray analysis. MET and HGF expression were examined in Affymetrix U133 Plus 2.0 array data from the 14 cell lines. Western blotting for total MET was performed in 14 cell lines. MET phosphorylation status was also determined in 11/14 cell lines using a human phospho-receptor tyrosine kinase antibody array (R&D Systems). Finally, we studied the sensitivity of 6 of the latter 11 lines to the investigational MET inhibitor, PHA-665752 (Pfizer).

Results: In 7/11 cell lines, there was prominent phosphorylation of MET. In 10/11 cell lines this was correlated with total MET by western blotting ($p<0.001$). The latter was also correlated with MET expression measured by microarray analysis (p=0.03). HGF transcript levels were elevated in only 1/14 cell lines (JMN, as previously reported) and did not correlate with MET RNA or protein levels or phosphorylation status. Two cell lines contained *MET* sequence alterations, both in the juxtamembrane domain and both previously described as germline polymorphisms: VAMT contained T1010I and Meso-9 contained R988C. Of 6 cell lines tested for sensitivity to PHA-665752 by MTT assay, Meso-9 had the lowest IC50 (0.625 uM), compared to 2.5 uM for VAMT and 1.3 uM for JMN, H2373, H2052, and H28. In these 6 lines, sensitivity to PHA-665752 was not correlated with the expression level of phosphorylated MET. The 55 patient samples showed known *MET* sequence polymorphisms (including T1010I in 3 cases) but only one novel non-conservative sequence alteration (G1137A) in the MET kinase domain in a single case. The germline vs acquired nature of the latter could not be determined due to lack of matching normal DNA.

Conclusions: Somatic mutations of *MET* are rare or absent in mesothelioma, contrary to some previous reports. The relative sensitivity of some mesothelioma cell lines to PHA-665752 appears to be based on non-mutational activation of HGF/MET signaling but only the findings in the JMN cell line support autocrine stimulation.

1522 Validation of Chromogenic In Situ Hybridization for Detection of EGFR Copy Number Amplification in Non-Small Cell Lung Carcinoma

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Background: A subset of patients with non-small cell carcinoma of the lung (NSCLC) has abnormal gene copy numbers in the epidermal growth factor receptor (EGFR) gene, and the relative importance of this finding to patient outcome is an area of great interest. Fluorescence in situ hybridization (FISH), the standard methodology to detect EGFR copy number abnormalities in NSCLC, is limited by fluorescence instrumentation. Chromogenic in situ hybridization (CISH) is an emerging alternative technique using light microscopy. In this study we examine EGFR expression by immunohistochemistry (IHC) and compare CISH and FISH in tissue specimens from Taiwanese non-smoking women with NSCLC.

Design: Specimens were obtained from patients treated at the Kaohsiung Veteran General Hospital, Taiwan, from 1999-2004. Tissue was methanol-fixed, paraffin-embedded, and cut into 10 micron sections. Each case was subtyped into adenocarcinoma (n=6), squamous cell carcinoma (n =4), or mixed pattern (n=3). EGFR IHC (clone H11, 1:400, Dako, Carpinteria, CA) was performed on protease-pretreated slides. FISH was performed using a probe to the EGFR gene on chromosome 7p and a control probe to 7q. CISH was performed using the CISH Detection Kit and EGFR amplification probe (Invitrogen/Zymed, South San Francisco, CA). Normal bronchial epithelium was used as an internal control. The FISH and CISH results were independently interpreted by two investigators.

Results: Of the 13 cases examined, 8 displayed no or low-level amplification by both CISH and FISH (2-5 signals per cell). Two cases demonstrated high-level amplification by both techniques. Discordant results were observed in 3 cases, in which high copy number was identified by CISH with no or low- level amplification by FISH. Positive EGFR IHC was seen in 2 of 2 cases with high-level amplification and 7 of 11 (63%) of cases with no to low-level amplification.

Conclusions: In most cases, CISH can accurately assess EGFR copy number abnormalities in non-small cell lung carcinoma. Postive EGFR IHC may correlate with EGFR copy number amplification. In a minority of cases, CISH overestimates copy number as compared to FISH. When high-level amplification is detected by CISH, confirmation by FISH may be necessary.

1523 KRAS, EGFR and p53 Alterations in Bronchioloalveolar Carcinoma and BAC-Predominant Adenocarcinoma

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Background: Variable EGFR mutation rates have been reported for bronchioloalveolar carcinoma (BAC), with Japanese studies reporting a high frequency of EGFR mutation (up to 60%). These data suggest the importance of EGFR mutations in the pathogenesis of BAC in the Japanese population. However, the genetic changes in BAC spectrum tumors in the US population have not been well studied.

Design: 51 tumors from 45 cases of BAC or adenocarcinoma with predominant BAC features (AD/BAC, >50% BAC) were analyzed, including 9 cases having 2 to 3 synchronous tumors. Tumor DNA was extracted from paraffin sections and KRAS codon 12/13 mutations and EGFR exon 19 and exon 21 mutations were analyzed by DNA sequencing. p53 overexpression was evaluated by immunohistochemistry (IHC), and only ≥2+ nuclear staining in >10% of tumor cells was considered as positive.

Results: Of 51 tumors analyzed, 12 were BAC and 39 were AD/BAC. 17 (33%) had KRAS mutations and 12 (24%) had EGFR mutations in exon 19 (3 tumors) or exon 21 (9 tumors). KRAS and EGFR mutations were mostly mutually exclusive, with only 2 tumors showing both KRAS and EGFR mutations. p53 overexpression was observed in 16 tumors (31%). Comparing BAC and AD/BAC, BAC had a higher rate of KRAS mutation (50% vs. 28%), a slightly lower rate of EGFR mutation (17% vs. 26%), and was similar to AD/BAC in p53 overexpression (33% vs. 31%). Of the 45 patients, 41 were smokers and 4 were non-smokers. KRAS mutations were seen exclusively in tumors from smokers (17/45, 38%) and none from non-smokers (0/6). In comparison, EGFR mutation frequencies were similar in both (10/45 vs. 2/6). This study included 8 males (all smokers) and 37 females (33 smokers). EGFR mutations were common in women (12/39 tumors, 31%) but not in men (0/12 tumors, p=0.047). In contrast, KRAS mutations were more frequent in men (6/12, 50%) than in women (11/39, 28%), although the difference was not statistically significant (p=0.18).

Conclusions: More frequent KRAS mutation (33%) and less frequent EGFR mutation (24%) was observed in our population of BAC spectrum tumors than was reported in Japan. This is likely attributed to higher frequency of smoking in our study group, although ethnic influence cannot be excluded. Similar to previously reported in lung adenocarcinoma, KRAS mutations occurred primarily in tumors from smokers while EGFR mutation was more common in females and non-smokers, indicating no fundamental difference between the pathogenesis of BAC spectrum tumors and lung adenocarcinoma in general.

1524 Mutational Analysis Supports Independent De Novo Carcinogenesis in Multifocal Adenocarcinoma of the Lung with Bronchioloalveolar Features

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Background: There is mounting evidence that adenocarcinoma containing bronchioloalveolar carcinoma (BAC) component arises from BAC. Multifocal tumors with BAC components thus may represent synchronous primary tumors rather than metastasis, even when the tumor foci are histologically similar. Previous clonality studies designed to address this issue have been limited and inconclusive.

Design: 61 tumors from 27 cases with multifocal adenocarcinoma were evaluated for KRAS, EGFR and p53. Tumor DNA was extracted from paraffin sections and KRAS codon 12/13 mutations and EGFR exon 19 and exon 21 mutations were analyzed by DNA sequencing. p53 overexpression was evaluated by immunohistochemistry (IHC), and ≥2+ nuclear staining in >10% of tumor was scored as positive.

Results: 26 of 27 cases had synchronous tumors and 1 had metachronous tumors. 24 cases had 2 foci of adenocarcinoma, 2 with 3 foci, and 1 with 5 foci. 19 cases were of similar histology among the multifocal tumors; 20 cases had at least one tumor that was either BAC or BAC-predominant (>50%). KRAS mutations were seen in 12 cases (19 tumors, 31%) and EGFR mutations in 5 cases (8 tumors, 13%). All KRAS and EGFR mutations were exclusively detected in patients with a smoking history (23/27). All but one KRAS mutations were at codon 12. Two exon 19 and 6 exon 21 mutations were seen in EGFR. 12 of the 17 cases showed mutations in only one of the synchronous lesions. Two cases showed identical mutations in the synchronous tumors (1 KRAS and 1 EGFR), suggesting metastasis. However, the case with the same KRAS mutations also had an EGFR mutation in one of the two tumors, whereas the case with the same EGFR mutations had a KRAS mutation in one of the three tumor foci, implying tumor progression or independent carcinogenesis. Most significantly, 3 of 17 cases showed different KRAS mutations in the separate tumor foci from the same case, clearly indicating de novo carcinogenesis. p53 IHC was positive in 22/61 tumors, with concordant overexpression in 5 cases, concordant negative staining in 12, and discordant staining in 10.

Conclusions: KRAS mutations are more frequent than EGFR mutations in multifocal adenocarcinoma, with both types of mutation seen only in smokers in this series. Analysis of KRAS, EGFR and p53 alterations indicates de novo carcinogenesis rather than metastasis as the main mechanism of multifocal adenocarcinomas with a BAC component, which strongly supports the theory of field cancerization.

1525 Immunohistochemical Expression of Estrogen and Progesterone Receptors in Primary Pulmonary Neuroendocrine Tumors

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Background: The most recent WHO classification of pulmonary neuroendocrine tumors (NET) includes typical carcinoids (TC), atypical carcinoids (AC), small cell carcinomas (SCC), and large cell neuroendocrine carcinomas. Carcinomas with neuroendocrine (NE) features also arise in many extra-pulmonary sites, and lungs are a common site of metastasis. The distinction between primary and secondary pulmonary NETs is often based upon immunostains. Primary and secondary pulmonary NETs often express similar NE markers including neuron-specific enolase, synaptophysin, chromogranin and/or CD56. When the differential diagnosis of a NET includes primary pulmonary versus metastatic mammary carcinoma, which occasionally has NE differentiation or are overtly a small cell carcinoma arising in a breast, thyroid transcription factor-1 (TTF-1), estrogen receptor (ER) and progesterone receptor (PR) immunostains are performed to determine the primary. Not all pulmonary NETs express TTF-1, some breast small cell carcinomas express TTF-1 and breast carcinomas often express ER/PR. The purpose of this study was to determine if primary pulmonary NETs express ER and PR.

Design: Twenty-six primary pulmonary NETs, including TC (10), AC (6), SCC (10) were stained for ER and PR. Staining intensity (1+ to 3+) and percentage of cells (PC) were evaluated.

Results: Nuclear ER and PR staining was observed in all three types of pulmonary NETs with variable INT and PC (see table 1). ER and PR staining did not correlate with age or sex. In addition, endothelial ER and/or PR expression was identified in TC (5/10, 50%) and AC (1/6, 17%).

Table 1. ER and PR Expression in Pulmonary NET

	ER Expression	ER (INT)	ER (PC)	PR Expression	PR (INT)	PR (PC)
TC	2/10 (20%)	1+	5-50%	3/10 (30%)	1+ to 3+	5-15%
AC	5/6 (83%)	1+ to 3+	5-100%	1/6 (17%)	3+	less than 5%
SCC	6/10 (60%)	1+ to 2+	5-50%	3/10 (30%)	2+ to 3+	20%

Conclusions: A large proportion of pulmonary TC, AC, and SCC are positive for ER and/or PR. PR expression has a predominantly 3+ intensity in pulmonary NET. These data show that positive ER and/or PR staining does not exclude a primary pulmonary NET.

1526 Galectin-4 (LGALS4) Expression in Neuroendocrine Carcinomas and Carcinoid Tumors of the Lung: A Tissue Microarray Study

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Background: Galectin-4 (LGALS4) is s a member of the galectin family of glycan binding proteins and has been found to be expressed in many human malignancies predominately colorectal carcinomas and neural tumors. Galectin-4 expression in primary pulmonary neuroendocrine carcinomas and carcinoids has not been documented and was investigated in this study.

Design: Tissue microarrays were constructed from 46 small cell carcinomas (SCC), 54 typical carcinoid tumors (CAR) and 11 large cell neuroendocrine carcinomas (LNC) using three 1.0 mm punch samples of formalin-fixed paraffin-embedded tumor tissue from each case. Immunohistochemistry for Galectin-4 (1:100, Vector Laboratories) was performed on recut sections of the microarrays. The percentage of tumor cells with nuclear staining was scored as 1 = <33%, 2 = 33-66%, 3 = >66% and nuclear staining intensity was scored as 0 = negative, 1 = weak, 2 = moderate and 3 = strong.

Results: Only nuclear staining was present, no cytoplasmic staining was seen. None of the LCN stained with Galectin-4 (0/11). The SCC showed 36% of tumors (17/46) with weak positive nuclear staining of 33-66% of the cells. The carcinoid tumors showed moderate to strong nuclear staining in 33-66% of tumor cells in 72% of the tumors (39/54).

Conclusions: Galectin-4 expression in tumors of the lung has not been documented previously. Current study demonstrates positive nuclear staining in SCC and Carcinoid tumors and no staining in LCN. Galectin-4 may prove to be a useful marker in the differential diagnosis of primary pulmonary neuroendocrine malignancies especially in small biopsies or limited tissue samples. However, the clinical significance and impact on patient survival of positive staining is not known. Further studies are being undertaken to evaluate and address expression of Galectin-4 in neuroendocrine carcinomas and other primary lung malignancies.

1527 Evaluation of Gene Amplification and Protein Expression of Epidermal Growth Factor Receptor (EGFR) by CISH, FISH and IHC in Lung Adenocarcinoma (ADC)

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Background: EGFR has attracted clinical attention as a molecular target for therapy. In (ADC), its expression has been reported from 38 to 75%, but the frequency of EGFR gene amplification has been reported to be only 7.8% using FISH and 5% using CISH.

Design: Twenty-four tumor samples from Early Lung Cancer Action Program (ELCAP) screen-detected lung cancers, resected at New York Hospital, were evaluated for EGFR amplification using CISH, FISH and protein expression by IHC. Clinical and pathological data were recorded for each case. Amplification was diagnosed when a gene copy number of 6 or more was present in more then 50% of tumor cells. IHC staining was interpreted according to Dako EGFR phamDx interpretation recommended for colorectal cancer: tumor was positive when ≥ 1% of the tumor cells exhibited any complete or incomplete circumferential membranous staining above background level. Positive cases were further stratified by staining intensity: 1+ (weak), 2+ (moderate) and 3+ (strong). The immunostaining extent was graded focal (≤50 % of the cells) or diffuse (>50% of the cells). Samples with a 2 or 3+ staining intensity, either focal or diffuse, were considered to have EGFR overexpression.

Results: Fourteen patients were females and 10 males. Age ranged from 47 to 77 years (m=64). All patients had a history of smoking: 8 were currently smoking and 16 were former smokers. 22 cases were classified as ADC, mixed subtype and 2 as large cell carcinomas. Tumor size ranged from 8 to 48mm (m=13mm). Seventeen cases were stage T1N0M0, 5 were T2N0M0, 1 was T4N0M0 and 1 was T4N2M1. IHC was negative in 6 (25%) and positive in 18 cases (75%), diffuse in 8 (29.6%) and focal in 13 (48.1%). Staining intensity was 1+ in 3 (12.5%), 2+ in 9 (37.5%) and 3+ in 6 (25%) cases. EGFR was considered to be overexpressed (2+/3+) in 15 (62.5%). Expression was correlated with histology. Amplification by CISH was not detected in any case using standard criteria. FISH was performed in the one case that had the highest average gene copy number by CISH, and EGFR gene amplification was not found.
Conclusions: In this group of ADC, EGFR expression by IHC was uncoupled from gene amplification defined by CISH or FISH using standard criteria. This study, conducted with a population of smoking patients, suggests that other mechanisms, such as gene mutation, might have a role in EGFR overexpression.

1528 Histopathological, CT and PET Scan Correlation of Pulmonary Lesions Identified in Patients Undergoing Surveillance for Extrapulmonary Malignancies

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Background: Pulmonary lesions found on surveillance CT scan in patients with extrapulmonary malignancies could represent non neoplastic changes, metastatic tumors or a new primary. Several publications cite algorithms based on size, CT and PET features to triage patients, so that invasive procedures are restricted to patients with a high probability of malignancy. Observations contrary to these algorithms (i.e. malignancy in a patient with non-suspicious CT and PET negative lesion) led us to correlate the CT and PET results in a cohort of patients with known extrapulmonary primary tumors.
Design: Retrospective data of 235 patients with known extrapulmonary primary tumors in whom secondary pulmonary lesions were identified during surveillance were included. The CT scans were classified as suspicious, non-suspicious or indeterminate for malignancy. CT scans classified as suspicious or indeterminate were considered as positive for statistical analysis. All lesions with a standard uptake value of > 2.5 were considered PET positive. Sensitivity, specificity, negative predictive values were calculated for each procedure. The gold standard for determining sensitivity of radiologic procedure was a diagnosis based on histopathologic analysis. If no surgery was performed, cases were considered benign if no lesion growth was identified on follow-up CT scans.
Results: The lesions ranged from 0.5 to 15 cm (mean 3.3 cm, 129 were < 3 cm). Malignancy was found in 83% lesion which were less than 3 cm, and in 63% lesions more than 3 cm. There were 204 cases with malignancy (165 primary, 27 metastatic tumors and 12 of indeterminate origin); 31 cases were non malignant. CT scan results were positive in 177 cases [sensitivity 54%, specificity 61% and negative predictive value 19%]. PET scan was positive in 106 cases (96/120 primary tumors and 5/17 metastatic tumors) [sensitivity 69%, specificity 65% and negative predictive value 22%].
Conclusions: Compared to published studies, both CT and PET scan were only moderately sensitive with a low negative predictive value. Spiculated lesions on CT characteristic of malignancy were identified only in 1/27 metastatic tumors. Additionally, PET scan was positive in only 29% metastatic tumors. Our results suggest that a multidisciplinary approach should be used in evaluating pulmonary lesions in high risk populations with high pretest probability of malignancy.

1529 Differences in Growth Signaling Pathway Activation in Pulmonary Small and Non-Small Cell Carcinomas – An Immunohistochemical Study

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Background: Small cell (SCLC) and non-small cell carcinomas of the lung (NSCLC) use different pathways to regulate growth and escape apoptosis. Our study aims to elucidate differences and similarities.
Design: Tissue microarrays were produced for SCLC (31 cases), adenocarcinomas (AC; 86), squamous cell carcinomas (SQC; 68), and large cell carcinomas (LC; 92), including sarcomatoid carcinomas (SC; 13) . An average of 5 punches of tumor tissue and 1 punch of normal lung tissue were taken from paraffin blocks. Immunohistochemistry was performed using commercially available antibodies, representing several pathways from the receptor/ligand site to the transcription factor level. A mean value for percentage and intensity of stained tumor cells was obtained and statistically evaluated. A network analysis for relationships between the different proteins was performed. Antibodies used: Tie2, VEGF, TGFa, EGFR1, MAP4K1, ERK2, ELK1, cMyc, cFos, cJun, CREB, IGF-I, IGF1Ra, IGF1Rb, IGF-II, PI3K, Akt, mTOR, Paxillin, AMPK, EBP1, Harmartin, Tuberin, CC10, p70S6K, PCNA, CyclinD1, cMet, PDGFRa, PDGFRb, GAB1, GRB2, SRC, STAT1, STAT3, STAT5, NFkB, b-Catenin, E-Cadherin, GSK3, JAK1, Survivin, FGFR3, MeCP2.
Results: SCLC and LCNEC have many deregulated pathways in common. In both SRC and NFκB is upregulated. The epidermal growth factor receptor (EGFR) pathway is upregulated in AC and SQC, but downregulated in SCLC. The hepatocyte growth factor-cMet pathway is high in SCLC, but low in NSCLC. Some of the binding proteins and transcription factors are differently regulated, such as low Gab1 and GRB2 in AC and SQC, but high in SCLC. MeCP2, a protein specifically binding to methylated DNA is high in AC, but low in all the other carcinoma types. Although the PDGF-receptor-α and β pathway is upregulated in almost all pulmonary carcinomas, the downstream factors STAT 1, 3, and 5, as well as Cyclin D are differently activated in SCLC and NSCLC.
Conclusions: In NSCLC (including LCNEC) the most important pathway is the MAP4K-pathway, whereas SCLC mediate their proliferation via c-Met. The high prevalence of apoptotic figures particularly in SCLC might be explained by the overexpression of NFκB. Our findings suggest that in LCNEC the therapeutic approach might be similar as in SCLC.

1530 Autocrine Signaling of the CXCR4-CXCL12 Axis in Non-Small Cell Lung Carcinomas

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Background: The CXCR4 receptor and its ligand CXCL12 have been implicated in metastasis of several carcinomas. CXCL12 has been proposed to function in a paracrine manner, with stromal cells producing the ligand and tumor cells the receptor. The expression pattern of CXCL12 has been proposed to explain patterns of metastases, with receptor-expressing tumor cells homing to organs that express the ligand. In this study, we examined the expression and localization of both CXCR4 and CXCL12 in various lung cancers.
Design: Using lung tumor arrays, we examined the expression and localization of CXCR4 and CXCL12 in archival paraffin embedded small cell (SCLC) and non-small cell lung carcinomas (NSCLC) by IHC. Each marker was evaluated by two pathologists and graded for both percentage of expressing cells and intensity of the expression.
Results: 10 SCLCs, 21 large cell carcinomas, 31 squamous cell carcinomas, and 22 adenocarcinomas were analyzed. All tumors showed at least focal expression of CXCR4 and most showed extensive nuclear and cytoplasmic expression. Interestingly, there was significant variation in the expression of the ligand. None of the small cell carcinomas expressed the ligand, suggesting that the CXCR4-CXCL12 signaling axis may function in a paracrine, metastasis-promoting manner in SCLCs. However, only 4 of 21 (19%) large cell, 9 of 31 (29%) squamous cell, and 1 of 22(4%) adenocarcinomas lacked expression of the ligand. Indeed, 10 of 21 (48%) large cell, 12 of 31 (39%) squamous, and 18 of 22 (82%) adenocarcinomas revealed extensive and intense staining for the ligand, while 7 of 21 (33%) large cell, 9 of 31 (43%) squamous, and 3 of 22 (14%) adenocarcinomas showed focal staining for the ligand. Thus, in 60 of 74 (81%) of NSCLCs, the CXCR4-CXCL12 axis may function in an autocrine, rather than paracrine, manner ($p < 0.0001$).
Conclusions: As previous described in other carcinomas, the CXCR4-CXCL12 signaling axis likely promotes metastasis of SCLC in a paracrine manner, as the tumor cells express the receptor but not the ligand. Surprisingly, we found that most NSCLCs express both the receptor and the ligand, suggesting that, unlike other carcinomas, NSCLCs utilize a CXCR4-CXCL12 autocrine signaling loop. Interestingly, very aggressive SCLCs lacked expression of the ligand, while the relatively less aggressive NSCLCs frequently expressed the ligand, suggesting that autocrine, rather than paracrine, signaling through the CXCR4-CXCL12 axis could hinder metastasis.

1531 Advancing Edge vs. Center of Non-Small Cell Lung Carcinoma: Assessment of Microvascular Density (MVD)

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Background: Angiogenesis is essential for sustained growth, invasion and metastasis of non-small cell lung carcinoma (NSCLC) and has led to the development of antivascular therapeutics. A better understanding of the temporal and spatial patterns of angiogenesis in NSCLC would aid in the prediction of stage and tumor growth patterns prior and subsequent to antiangiogenic therapy. We evaluated the vascular distribution within the tumor advancing edge vs. center in Stage IA and Stage III NSCLC.
Design: We evaluated 20 cases of NSCLC (Stage IA, n=10 and Stage III, n=10) using CD31 IHC on formalin-fixed, paraffin-embedded tumors. MVD was determined on scanned digital images using an automated cell imaging system (ACIS, Clarient). The areas with the highest vascularization were chosen at the center of the tumor tissue section and at one advancing tumor edge. The MVD in at least four 400X fields was assessed and averaged.
Results: The CD31+ MVD was higher in Stage III compared to Stage IA in the intratumoral center ($p=0.0129$) and tumor edge ($p=0.0023$). In Stage IA NSCLC, the CD31+ MVD was higher in the edge compared to the center of the tumor ($p=0.0375$). In Stage III NSCLC tissue sections the CD31+ MVD was higher (2 to 3 fold) in the tumor edge compared to the center in 30% of cases ($p<0.05$ for each of 3 cases). However, in 70% of Stage III cases, the MVD was similar at the edge and center of the tumor section ($p=0.4761$). The 30% of Stage III cases in which the MVD increased 2 to 3 fold in the tumor edge had a relatively low MVD in the tumor center as compared to the center of the remaining 70% of Stage III cases ($p= 0.00048$).
Conclusions: As expected, there was a significantly higher MVD in Stage III tumors as compared to Stage IA NSCLC. In Stage IA NSCLC, the tumor edge had a higher CD31+ MVD compared to the tumor center, which may reflect a peripheral infiltrative tumor growth pattern stimulating angiogenesis. However, it appears that due to the inherently high MVD in the center of Stage III NSCLC cases overall, there is no further increase in CD31+ MVD at the peripheral edge of the tumor compared to the center. Alternatively, due to the larger size of tumors in the Stage III cases (which we evaluated on a single slide), identifying the infiltrative tumor edge with the highest MVD may require more extensive tissue sampling. Our studies illustrate a spatial difference in angiogenesis in Stage IA NSCLC and in comparison, either a higher level of angiogenesis at the peripheral edge or throughout the tumor in Stage III NSCLC.

1532 Mutation and Expression Analysis of EGFR in Primary Pulmonary Salivary Gland Type Carcinomas

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Background: EGFR expression is believed to be present in a subset of lung tumors and mutational status of the EGFR gene is thought to be associated with sensitivity to tyrosine kinase inhibitors. Since EGFR biology in pulmonary salivary gland type carcinomas is not well characterized, the goal of this study was to elucidate the EGFR mutational status and protein expression in these uncommon pulmonary tumors.

Design: DNA from formalin fixed paraffin-embedded tissue sections from 10 low-grade mucoepidermoid carcinomas (MEC) and 10 primary adenoid cystic carcinomas (ACC) were extracted by NDME method (Lab Invest. 2005, 85(11):1416-28) and analyzed for deletion in exon 19 by fragment analysis on ABI 310. Tissue microarray (TMA) based samples of 14 ACC and 14 MEC were studied immunohistochemically for EGFR and phosphorylated downstream molecules including p-STAT3, p-Akt, p-Erk1/2 and p-mTOR. Correlation analysis was performed between clinical and molecular/IHC variables.

Results: Eleven of 20 salivary gland type carcinomas, including 70% of ACC and 40% of MEC, revealed exon 19 deletions. ACC were positive for EGFR in 64.3%, p-ERK1/2 in 28.6% and p-STAT3 in 7.1%, but negative for p-mTOR and p-Akt. MEC were positive for EGFR in 14.3%, p-mTOR in 21.4%, p-ERK1/2 in 21.4%, p-Akt in 35.7% and p-STAT3 in 21.4%. There was no correlation between EGFR mutational status and age, sex, EGFR expression or studied downstream molecules in either group.

Conclusions: The pulmonary salivary gland type carcinomas demonstrate frequent exon 19 mutations of EGFR gene, but no association of the mutational status with IHC EGFR expression. This molecular aberration is more common in ACC than in MEC. While ACC express EGFR more commonly than MEC, the latter has a tendency to show upregulation of downstream molecules p-Akt and p-mTOR. Whether the mutational status of this subset of tumors is important in prognosis and is predictive of response to novel chemotherapeutic agents remains to be investigated.

1533 EU-US Pathology Panel for Uniform Diagnosis in Randomised Controlled Trials for CT Screening of Lung Cancer. Learning Phase in Diagnosis of Early Lung Cancer and First European RCT Cases

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Background: RCT for lung cancer screening using HRCT are now underway. In order to allow future effective comparison of the different trials as well as strengthening conclusions based upon the analysis of larger data sets, uniformity and consistency of pathology diagnosis is essential. The aim of this study is to determine the effectiveness of the learning process and application in this difficult area of diagnostic pathology.

Design: Eight pathologists received two CD-ROMs with digital images of 30 cases each. After diagnosing the first series, selected background reading was provided. Kappa scores were calculated for each pathologist and each category and compared to the consensus score. In addition 73 European RCT read from a web server.

Results: The readings of the first series showed a moderate agreement kappa score: mean and standard deviation for "8"(all 8 categories) and "2" categories was 0.53 ±0.05 and 0.65 ±0.04, respectively. The kappa "2" score distinguished between categories denoting benign and malignant lesions. The second series resulted in a good agreement kappa score: mean and standard deviation for "8" and "2" categories was 0.65 ±0.06 and 0.81 ±0.02. 73 European RCT cases were read, of which 56 (77%) and 12 (16%) cases were unequivocal malignant and benign, respectively. In 5 cases ambiguity between benign and malignant was present. The indeterminate cases require further judgment on a multi-headed microscope.

Conclusions: Screen-detected cases pose particular problems for pathologists and a trained pathology panel serving RCT is likely to lead to more consistent and accurate tissue diagnosis. Panel reading is obligatory for uniform pathology diagnosis across RCT CT lung cancer screening trials.

1534 Metastatic Malignant Pleural Mesothelioma in the Mediastinal Lymph Node: A Clinico-Pathologic-Radiologic Study of Seven Cases

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Background: Malignant pleural mesothelioma metastatic to lymph nodes is reportedly rare. Histopathological diagnosis in such biopsies may not be straightforward. We reviewed 7 cases to define the importance of clinical, pathological and radiologic data in the diagnosis of metastatic mesothelioma in the mediastinal lymph nodes.

Design: Seven cases of metastatic mesothelioma in mediastinal lymph node were retrieved from the files of the authors, and slides, clinical history and imaging data were reviewed. In all cases, immunohistochemistry was performed for calretinin, CK5/6, p63 and CD5; in selected cases, immunohistochemistry was performed for epithelial markers and TTF-1 as well.

Results: All 7 patients had pleural effusion. In 5, diagnosis of malignant pleural mesothelioma was previously known (4 cases) or made at the time of mediastinal biopsy (1 case). Initial presentation in the remaining two cases was that of mediastinal lymphadenopathy. In one case, initial diagnosis of mesothelial cell inclusions was made based on light microscopy, immunohistochemistry and negative CT findings. Subsequent fine needle aspiration of an enlarged cervical lymph node found atypical mesothelial proliferation, suspicious for metastatic mesothelioma. Video-assisted thoracoscopy showed small nodules on the visceral surface, pleural biopsy was diagnosed as malignant epithelioid mesothelioma, and the mediastinal lymph node biopsy was reinterpreted as metastatic mesothelioma. In the last case, mediastinal lymph node biopsy showed malignant epithelioid cell proliferation; immunohistochemistry showed tumor positive for caltretinin, CK5/6, p63 and CD5 (cytoplasmic), and negative for epithelial markers and TTF-1. Based on immunohistochemstry and a CT finding of pleural thickening, the diagnosis of metastatic mesothelioma was considered most likely. Because p63 and CD5 positivity are more typically observed in thymic tumors, electron microscopy (EM) was done which showed long microvilli characteristic of malignant mesothelioma.

Conclusions: Malignant pleural mesothelioma may present as mediastinal lymphadenopathy with pleural effusion, and integration of clinical, pathological, and radiologic data is essential in making the diagnosis of pleural mesothelioma metastatic to mediastinal lymph nodes. Caution should be used when diagnosing mesothelial cell inclusions in mediastinal lymph node.

1535 CXCR4 Expression in Stage I, Stage II and Multifocal Non-Small Cell Lung Carcinomas

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Background: CXC chemokine receptor 4 (CXCR4) expression levels have been correlated with metastatic spread of malignant neoplasms. CXCR4 has been found in higher levels in metastatic non-small cell lung carcinomas (NSCC) relative to non-metastatic tumors. The aim of the current study was to compare the expression of CXCR4 in stage I, stage II and multifocal NSCCs.

Design: Unifocal stage I, unifocal stage II and multifocal NSCC cases were identified retrospectively from institutional records. All tumors were stained with CXCR4 antibody by immunohistochemistry, and the intensity of staining and proportion of cells staining were quantified. Tumors were classified as either weakly staining (low intensity or <50% of malignant cells) or strongly staining (intense staining in ≥ 50% of malignant cells).

Results: All tumors stained either weakly or strongly for CXCR4. Most stage I tumors were weakly positive, while most stage II tumors were strongly positive (see table). Multifocal tumors exhibited significantly higher CXCR4 expression (14/18 cases strongly positive) relative to unifocal stage I carcinomas (3/10, p = 0.02). Five out of six tumors from three patients with multifocal disease and lymph node metastases stained strongly positive for CXCR4. Among metastatic tumors, there was concordance in CXCR4 expression between the lymph node metastasis and the primary tumors in most cases available for examination (6/7).

CXCR4 staining in stage I, stage II and multifocal NSCCs

CXCR4 Staining		
	Weak	Strong
Stage I (n=10)	7 (70%)	3 (30%)
Stage II (n=9)	1 (11%)	8 (89%)
Multifocal (n=18)	4 (29%)	14 (71%)

Conclusions: Strong immunohistochemical staining for CXCR4 in NSCCs was associated with significantly greater probability of multifocality or lymph node metastases (i.e., higher stage disease) relative to weakly staining tumors. It may therefore provide prognostic information and help to identify patients who require adjunctive therapy.

1536 Molecular Evidence for Common Clonal Origin of Multifocal Lung Cancers

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Background: Lung cancer is the most common cause of cancer death in the United States. Multiple anatomically separate but histologically similar lung tumors are often found in the same patient. Little data is available concerning clonality in multiple lung tumors.

Design: This study included 39 tumors from 19 female patients (14 patients with non-small cell carcinomas and 5 patients with carcinoid/atypical carcinoid tumors) who underwent lobectomy or pneumonectomy for lung epithelial tumors. Histologic diagnosis in these 39 tumors was as follows: 22 adenocarcinomas, 6 squamous cell carcinomas, 2 large cell carcinomas, and 9 carcinoid tumors. All patients had multiple tumors (two to three) involving one or both lungs. Genomic DNA was prepared from paraffin-embedded tissue sections using laser-capture microdissection. LOH studies were performed using a panel of 5 polymorphic microsatellite markers at chromosome 9p21 (IFNA, D9S171), 17p13 (TP53), 3p14-21 (D3S1766) and 4q25-32 (D4S408). In addition, X chromosome inactivation status was examined.

Results: Eighteen of 19 cases (95 %) showed loss of heterozygosity in at least one of the five polymorphic microsatellite markers (ranging from one to three markers). Concordant LOH patterns between each coexisting tumor were seen in 16 of 19 cases (84%). A concordant pattern of nonrandom X chromosome inactivation in the coexisting tumors was seen in 12 of 16 (75%) of informative cases; while a discordant pattern of nonrandom X chromosome inactivation was seen in 1 of 16 cases (6%); the remaining 3 cases (19%) showed random X chromosome inactivation.

Conclusions: Our data indicate that multifocal lung cancers often have a common clonal origin. This suggests that local and regional parenchymal metastasis may play an important role in the spread of lung cancer.

1537 Immunohistochemical Expression of Protein Kinase C (PKC) ß in Non Small Cell Lung Cancer (NSCLC) and Mesothelioma

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Background: The PKC family of serine-threonine protein kinases has been shown to be involved in the control of tumor cell growth, survival, and progression. Tumor-induced angiogenesis requires activation of PKCs, particularly PKC ß, through vascular endothelial growth factor (VEGF) pathways. Stimulating the VEGF receptor (VEGFR) initiates a cascade resulting in tumor angiogenesis and neovascularization. PKC ß appears to be a major down-stream signaling protein for VEGFR. This makes PKC ß an attractive target in treatment of malignancies, including that of NSCLC.

Design: 111 NSCLCs [52 adenocarcinomas (AC), 34 large cell (LC), 25 squamous (SCC)] and 24 malignant mesotheliomas (MM) arranged in tumor microarrays (TMAs) were stained immunohistochemically for PKC ß-1 and 2. Sections of uninvolved lung, tumor center, and invasive fronts were included for AC, LC, and SCC. The MM TMAs included tumor and uninvolved lung. Nuclear staining was analyzed manually and scored for intensity (1+, 2+, 3+) and distribution (1=1-10%, 2=11-50%, 3=51-100% of tumor staining). Clinical information was available for the AC, LC, and SCC: gender data was available for 110/135, race data for 109/135, smoking data for 106/135, and survival data available for all 74 patients in the SCC and AC groups.

Results: PKC ß-1 stained 2+/3+ in 100% of MM, 96% of AC, 91% of SCC and 64% of LC. For PKC ß-2, 81% of AC, 38% of SCC, 39% of LC and 22% of MM stained 2+/3+. The majority of tumor centers and their respective advancing edges corresponded in staining intensity. For PKC ß-1, staining concordance was 95% in AC, 91% SCC, and 74% LC. Similarly, for PKC ß-2, staining concordance was 81% in AC, 84% SCC and 69% LC. PKC ß-1 and 2 staining in the tumor center was not significantly different by clinical stage (TNM), patient gender, race or smoking status. Survival data for the 74 AC and SCC patients showed that the 5% who were negative for PKC ß-1 in tumor centers had significantly increased survival time (124 days versus 1258 days, p=0.0003). There was no significant difference in survival by PKC ß-2 expression.

Conclusions: PKC ß-1 is strongly expressed in the majority of AC, SCC, LC and MM. PKC ß-1 expression is an adverse prognostic indicator in AC and SCC. PKC ß-2 showed strong staining in AC. PKC ß is expressed not only in the tumor center, but also in the advancing edge. Therefore, PKC ß may be an important target in improving the therapeutic strategy in selected NSCLC.

1538 Immunohistochemical Detection of XIAP and p63 in Thymic Hyperplasia and Thymomas

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Background: The classification and biological behavior of thymomas have long been a confusing issue. Expression of p63 has been reported in human thymus and thymic epithelial tumors. A recent study reported XIAP expression in normal human tissues expression in normal human thymus. However, there has not been any study of XIAP in thymic hyperplasia and thymomas. We performed an immunohistochemical survey of XIAP and p63 in human thymic hyperplasia and thymomas.

Design: Twenty two (22) formalin fixed paraffin embedded tissue blocks including 11 cases of thymic hyperplasia, 11 thymomas of which 4 were encapsulated and 7 were invasive were subjected to citrate based antigen retrieval then incubated with monoclonal anti-XIAP (# 610763, BD Biosciences, San Jose, USA) 1:250, 4 C for 72 hrs and developed using EnVision-Plus reagents (Dako) and diaminobenzidine as chromagen; and with monoclonal anti-p63 (4A4, Santa Cruz), 1: 4000 with 0.1% bovine serum albumin and 5% non-fat dry milk in room temperature for 16 hrs. Granular or heterogeneous cytoplasmic staining for XIAP and nuclear staining for p63 were considered positive.

Results: The thymic epithelial cells in near all cases of hyperplasia were negative for XIAP but positive for p63 with one exception in which rare spindle shaped epithelial cells were positive for XIAP. In contract, nine out of ten thymomas (81.8%) were XIAP positive with focal positivity in 3 out of 4 (75%) encapsulated and variable positivity (from focal/weak to strong/diffuse) in 6 out of 7 (86%) invasive thymomas. p63 was diffusely positive in epithelial elements, in comparison to which XIAP positivity varied from focal to diffuse.

Conclusions: Increased expression of XIAP suggests a possible role in the pathogenesis of thymoma. In contrast, p63 is consistently positive in both non-neoplastic and neoplastic thymic epithelium. Acknowledgement: Supported by a generous bequest from the Estate of Hilda Leveen (D.E.B.)

1539 K Homology Domain Containing Protein Overexpressed in Cancer (KOC) Is Highly Expressed in Small and Large Cell Neuroendocrine Carcinomas but Not in Carcinoid Tumors of the Lung

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Background: K homology domain containing protein overexpressed in cancer (KOC), also known as L523S and IMP3, is a member of the insulin-like growth factor (IGF) mRNA-binding protein (IMP) family and is expressed during embryogenesis and in certain malignancies. It promotes tumor cell proliferation by enhancing IGF-II protein expression. However, the expression of KOC and IGF-II has not been investigated in lung neuroendocrine tumors (LNETs).

Design: Forty-six surgically resected LNETs, including 16 typical carcinoids (TC), 6 atypical carcinoids (AC), 14 large cell neuroendocrine carcinomas (LCENC) and 10 small cell carcinomas (SCLC), were immunohistochemically studied using antibodies against KOC/L523 and IGH-II. Cytoplasmic staining was considered positive, and the percentage of positively stained tumor cells was recorded. The immunostaining intensity was graded as weak, moderate, or strong. A p value of <0.05, as determined by Fisher's exact test, was considered statistically significant.

Results: Nine of 10 (90%) SCLCs and 9 of 14 (64%) LCNECs showed strong and diffuse cytoplasmic staining for KOC, with positive staining seen in >90% of the tumor cells. The remaining one case of SCLC and 5 cases of LCNEC also exhibited a variable degree of KOC immunoreactivity. Although SCLCs tended to more frequently express KOC in a strong and diffuse pattern than LCNECs, the difference did not reach statistical significance (p>0.05). No positive KOC staining was detected in any of the 16 TCs. Five ACs also showed a complete lack of immunoreactivity. In only one AC was weak staining observed in areas exhibiting oncocytic change. IGF-II expression was detected in all 46 LNETs including TCs and ACs, with similar staining patterns.

Conclusions: 1. KOC is highly expressed in high grade LNETs (SCLCs and LCNECs) but not in low grade carcinoid tumors, suggesting KOC plays an important role in the regulation of biological behavior of high garde neuroendocrine carcinomas. 2. IGF-II is ubiquitously expressed in LNETs including those lacking KOC expression, suggesting a more general role in tumorigenesis and an involvement of more complex regulatory mechanisms in the control of IGF-II expression.

1540 Immunohistochemical Differential Expression in Lung and Breast Cancers

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Background: The risk of developing a second primary cancer is increased in patients with breast cancer, and the lung is one of the major sites involved. Furthermore, the lung is the major metastatic site for breast cancers. A distinction between metastatic breast cancer and primary lung cancer is often histologically difficult, and both show overlapping immunophenotypes such as CK7+/CK20- in a majority of cases. The degree of difficulty increases with poorly differentiated tumors. Negative TTF-1 expression and focal ER reaction in occasional lung adenocarcinomas further adds to the difficulty. We conducted a panel of immunohistochemical markers in order to observe the differential expression in both cancers.

Design: 23 cases of poorly differentiated non-small cell lung carcinoma comprised of 16 cases of adenocarcinoma and 7 of non-keratinizing squamous cell carcinoma, and 23 cases of poorly differentiated breast ductal carcinoma (grade III in Nottingham system) were studied. Immunostains for PE-10 (surfactant apoprotein A), CC16 (Clara cell protein 16) , DC-LAMP (CD208), TTF-1, ER, PR, mammaglobin, GCDFP-15 and Her2/neu were performed. The extent of staining was graded as focal (<50%) or diffuse (>50%).

Results: Mammaglobin and GCDFP-15 were expressed to a variable extent in 57% and 57% of breast cancers, respectively. ER was positive in 48% of the cases, with the expression being focal and diffuse in 13% and 35% of cases, respectively. Only four cases of breast cancers (17%) were negative for all three markers. PR expression was focal in one-third of the cases, and Her2 expression was focal and diffuse in 48% and 42% of cases, respectively. Lung markers were completely negative in all the breast cancers except for CC16 which was focally reactive in 13% of cases. In contrast, mammaglobin, GCDFP-15 and PR were negative in all of the cases of lung cancers, and ER was only focally expressed in one lung cancer. Her2 expression was focal and diffuse in 30% and 9%, respectively. Among the lung markers, the highest expression was seen in TTF-1 (48%), followed by PE-10 (22%), CC16 (13%) and DC-LAMP (4%). Lung cancers negative for TTF-1 were also negative for all other lung markers.

Conclusions: 83% of poorly differentiated breast ductal carcinomas were positive for mammaglobin and/or GCDFP-15 and/or ER (diffusely). Thus, when a metastasis from breast cancer is suspected for a tumor in the lung, a panel of mammaglobin, GCDFPD-15 and ER is recommended. As expected, TTF-1 is the most sensitive lung marker compared with other immunostains tested.

1541 Cytokine Release from Alveolar Macrophages in Idiopathic Interstitial Pneumonia

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Background: Idiopathic interstitial pneumonia (IIP) is a group of interstitial pneumonias with unknown causes, classified as different subgroups according to the histological characteristics. Alveolar macrophages (AM) are able to release various cytokines and play an important role in the inflammatory and fibrotic process of IIP. However, the production of cytokines by AMs in the subgroups of IIP is still unclear.

Design: We measured the release of TNF-α, TGF-β, IL-1β, IL-6, IL-10, IL-12 and IL-18 from bronchoalveolar lavage (BAL) macrophages in 9 patients with cryptogenic organising pneumonia (COP), 13 patients with non-specific interstitial pneumonia (NSIP), 5 patients with respiratory bronchiolitis interstitial lung disease (RBILD), 4 patients with desquamative interstitial pneumonia (DIP), 19 patients with idiopathic pulmonary fibrosis (IPF) and 10 controls. AMs were cultured for 24h with RPMI medium alone or with lipopolysacharide (LPS) 100ng/ml. Cytokines in the supernatants were measured by ELISA.

Results: The spontaneous levels of TNF-α, TGF-β, IL-1β, IL6, IL-10, IL-12 and IL-18 released from BAL macrophages were significantly higher in COP than in IPF and controls (p<0.05 or <0.001 respectively), and except for IL-18 also significantly higher in NSIP than in IPF and controls (all p<0.05) . The production of these cytokines with or without LPS stimulation was highest in COP, followed by NSIP, and lowest in DIP/RBILD and IPF.

Conclusions: This study demonstrates that the different subgroups of IIP show different levels of cytokine release by BAL macrophages. This may be associated with the pathogenesis of IIP and may contribute to the different phenotypes and prognosis of the subgroups.

1542 Protein Expression and Gene Amplification of Epidermal Growth Factor Receptor in Non-Small Cell Lung Cancer; Correlation with Chemoresponse to Gefitinib Therapy

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Background: The human epidermal growth factor receptor (HER) family of receptor tyrosine kinase has been demonstrated to be overexpressed in the majority of NSCLC, which led to the clinical development of molecular therapies targeting epidermal growth factor receptor (EGFR). Among the various small molecule inhibitors of tyrosine kinase, gefitinib has been approved for clinical use in patients with previously treated advanced NSCLC. It has shown dramatic effectiveness in some patient subsets, including women, never-smokers, adenocarcinomas, and East Asian descents. However, predictive molecular markers are yet to be determined.

Design: EGFR protein overexpression by immunohistochemistry and gene amplification by chromogenic *in situ* hybridization (CISH) were analyzed in biopsy specimens from 30 patients with advanced NSCLC. After failure of first-line treatment, all had received gefitinib. Time to progression (TTP) and overall survival (OS) were correlated with EGFR status.

Results: EGFR overexpression was detected in 47% (14/30) of the tumors. Of these, EGFR gene amplification was found in 62% (8/13). EGFR expression was not associated with outcome. Median TTP was 5 months and OS was 29 months. Although there was no difference in OS between EGFR-positive and –negative groups, TTP was prolonged in EGFR-positive patients (8.4 months vs. 5.7 months; p=0.259), and in female patients (9.7 months vs. 2.9 months; p=0.007). Three of 8 (38%) EGFR CISH positive patients demonstrated disease control versus one of five (20%) patients in CISH-negative group.

Conclusions: Increased EGFR gene copy number was associated with improved response to gefitinib therapy. Since EGFR overexpression was accompanied predominantly, but not exclusively, by gene amplification, it might be important to evaluate both EGFR expression and gene amplification in identifying patients most likely to benefit from gefitinib therapy. EFGR status along with EGFR downstream molecules need to be further investigated in a larger series of informative NSCLC cases.

1543 Clinical Significance of p-AKT Pathway in Non-Small Cell Lung Cancer (NSCLC)

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Background: Dysregulation of the AKT pathway is known to be important in human cancer with emerging data in NSCLC. However, little is known about the role of upstream PTEN and downstream mTOR, TSC2, ERK1/2, S6, and eIF4E. We sought to investigate these markers in a large series of NSCLC.

Design: We immunohistochemically stained a TMA with 300 NSCLC using antibodies to PTEN, p-AKT, p-mTOR, p-TSC2, p-S6, and p-eIF4 and p-Erk1/2. A score for each case was made based on distribution and intensity of staining to determine positive thresholds. Follow-up was available in 244 cases. Kaplan Meier survival analysis and chi-square statistics were made using SPSS 13.0.

Results: In NSCLC many correlations were found: positive PTEN correlated with positive AKT (p<0.001) and negative ERK1/2 (p=0.006), while negative PTEN correlated with eIF4E & S6 (p=0.020 & 0.012). Positive AKT correlated with positive mTOR (p=0.047) and negative eIF4E and S6 (p=0.025 & 0.011). Negative p-mTOR correlated with negative S6 and ERK1/2 (p=0.013 and 0.038). Negative TSC2 correlated with negative eIF4E and ERK 1/2 (p=0.011 & <0.001). Negative eIF4E and ERK1/2 correlated with negative S6 (p=0.007 and p<0.001). Survival correlations were found for all NSCLC with overexpression of eIF4E and S6 and patients whose tumors had overexpression of AKT and loss of PTEN (Table). The latter finding was mostly seen in squamous cell carcinoma. For adenocarcinoma significantly worse survival was seen in patients whose tumors overexpressed AKT and eIF4E. With squamous carcinoma overexpression of PTEN correlated with favorable prognosis.

Conclusions: Dysregulation of the AKT pathway is important in NSCLC with many interactions between downstream factors and prognostic correlations with eIF4E and S6.

Percent 5-Year Survival By Histology and Marker

Histology	Marker	Negative	Positive	p-value
NSCLC	eIF4E	46.2	23	0.005
	S6	45.3	22	0.003
	AKT+PTEN- vs Others	43.8	33.2	0.036
Adenoca	AKT	83.3	29.6	0.048
	eIF4E	42.4	23.5	0.019
Squamous	PTEN	33.5	53.3	0.046
	AKT+PTEN- vs others	47.2	33.3	0.023

1544 Folate Receptor (FR) Expression in Stage 1 and 2 Non-Small Cell Lung Cancers (NSCLC): Correlation with Patient Survival and Potential Target for Delivery of Therapy

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Background: NSCLC patients have a poor prognosis, with half of patients with stage 1 and 2 cancers dying of their disease despite tumor resection. Novel approaches aimed at new molecular targets are obviously needed. The folate receptor (FR) is often over-expressed on cancer cells, presumably to facilitate uptake of extra amounts of folic acid, a vitamin required for DNA synthesis. Because most normal cells express no FR, the receptor has emerged as an ideal molecular target for delivery of folate-linked chemotherapeutic agents. Except for ovarian cancer, data on the frequency of FR over-expression in human cancers are limited to a few tissues sections per cancer type. In a multi-institutional collaboration, we immunostained a NSCLC TMA with mAb343, a murine monoclonal antibody against FR-α previously shown to stain only frozen tissue sections (Int J Cancer, 1994) and evaluated these results with respect to patient 5-year survival.

Design: Formalin-fixed, paraffin-embedded sections of a TMA with 3 punches from each of 286 stage 1 and 2 NSCLC with 5-year survival follow-up were immunostained for mAb343. Immunopositivity in tumor cells was graded on a scale from 0 to 3 and averaged for the 3 punches from each tumor. Average score of 0 or1 was classified as weak expression, while average score of 2 or 3 was classified as strong expression. FR expression was compared to 5-year survival using Kaplan-Meier analyses, including by cell type (adeno, squamous, large cell) and tumor grade (low grade= grades 1 and 2, high grade=grades 3 and 4).

Results: 223/286 stage 1 and 2 NSCLC expressed FR. Strong expression was associated with longer survival (p < 0.05). This association was not correlated with cell type but was statistically significant for tumor grade. Strong FR expression was not associated with longer survival in low grade tumors (p=0.14). Strong FR expression was associated with longer survival in high grade tumors (p=0.045).

Conclusions: FR is over-expressed in 78% of NSCLC and provides a potential molecular target for receptor-targeted therapies. Strong FR expression is associated with 5-year survival in high grade NSCLC.

1545 The Prognostic Value of p53 and Ki-67 Expression in Thymic Neoplasm: A Clinicopathological and Immunohistochemical Study of 60 Thymic Tumors

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Background: To investigate the expression of p53 and Ki-67 in correlation with clinicopathological variables in a series of thymic epithelial tumors, and to establish prognostic values of these biological parameters.

Design: A total of 60 patients with thymic epithelial tumors were reviewed retrospectively. The histologic subtypes were based on the new World Health Organization classification, and the stage was determined according to the modified Masoka's staging system. Additional key clinical information including tumor size, patient survival, local disease recurrence, and treatment modality was also recorded. Immunohistochemical expression of nuclear p53 and Ki-67 was evaluated in relation to the clinicopathologic variables. A score of immunostain reactivity was given in each case by multiplying the intensity level by percentage of tumor cells at each intensity, and a score more than 30 was considered to be positive. Chi-square test and Mann-Whitney U tests were used for statistical analysis.

Results: Among 60 patients, male to female ratio was 1:1 and median age was 63 years. Nuclear expression of p53 and Ki-67 was identified in 29 of 60 (48%) and 30 of 60 (50%) cases, respectively. Ki-67 expression was significantly associated with the larger tumor size (p<0.05). p53 expression was significantly correlated to the higher tumor stage (p <0.01): all 6 of 6 stage IV tumors (100%) expressed p53, compared to 43% of stage I, II, and III tumors with p53 expression. The histologic classification of the tumor included 6 type A, 15 type AB, 8 type B1, 6 type B2, 17 type B3, and 8 type C, in an increasingly order of malignancy. Both p53 and Ki-67 expression was significantly related to these histologic subtypes with a greater degree of malignancy and poorer prognosis. p53 was expressed in 17 of 25 (68%) type B3 and type C tumors, compared to 12 of 35 (34%) positive cases of combined type A, AB, B1 and B2 (p<0.05). Similarly, Ki-67 was positive in 10 of 25 (40%) type B3 and type C tumors, whereas no case in type A was positive for this marker ((p<0.01).

Conclusions: Nuclear expression of p53 and Ki-67 was associated with high stage and more aggressive histologic subtypes of thymic epithelial tumors, suggesting these two markers may be of prognostic value for the thymic neoplasms.

1546 HIF-1, VEGF and NFKB in Primary Lung Hypertension (PH) in a Sheep Model

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Background: Identifiying the molecular mechanism underlying PH and the progression of the arterial structural changes will be helpful for understanding this rare disease. Therefore we have developed a unique sheep model for PH.

Design: Induction of air embolism in pulmonary circulation of 4 adult sheep via permanent pulmonary artery catheter was done for a period of 8 weeks at a rate of 6-80 ml/hour. Baseline values of hemodynamics and blood gases were measured and recorded daily. Infusion was terminated at week 8 and sheep were sacrificed at week 9. 5 tissue sections where obtained from each lobe of the lung and stained with hematoxilin and eosin, movat, trichrome, HIF-1 (1:2000, Abcam), osteopontin (1:100, Calbiochem), and NFkB (1:100, cell Signaling). For each immunostain a % of endothelial cell staining was scored as positive if 5% or more cytoplasmic (osteopontin), nuclear (NFkB and HIF-1) staining was seen.

Results: All animals met the PH criteria within 3 weeks. Baseline pulmonary artery pressure was 14mmhg, increase to > 25 at week 4, 35 at week 8, and 32-35mmHg at 9th week. Comparing with baseline, cardiac output was lower at completion and central venous pressure was higher while heart rate, artery pressure and blood gases remained normal. In all 4 sheep the lungs revealed severe pulmonary arteriopathy in all pre and intra-acinar arteries with marked cellular intimal thickening, consistent with chronic PH. The intimal thickening was due proliferation of endothelial cells, hypertrophy and proliferation of fibroblasts, myofibroblasts and smooth muscle cells. The endothelial proliferation was positive for VEGF and negative for NFKB and HIF-1.

Conclusions: The microscopic features seen in each sheep where those of pulmonary arteriopathy with intimal thickening (cellular). The endothelial proliferation highly express VEGF but are negative for HIF-1 and NFKB.Therefore overexpression HIF-1 and NFKB are not important mechanisms in the marked endothelial proliferation seen in these type of PH.

Quality Assurance

1547 Comparison of Cytotechnologist and Pathologist Interpretation of HER-2/*neu* Expression in Breast Carcinoma

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Background: Accurate assignment of HER2/*neu* status is essential to clinical decision making in the treatment of breast cancer. Immunohistochemistry (IHC) is commonly used assay for evaluation of HER-2/*neu* status. Despite criteria for interpretation of staining results for HER-2/*neu*, the determination of staining intensity and the percentage of complete membrane staining is subjective. At our institution, IHC HER2/*neu* specimens are pre-screened by a cytotechnologist (CT) prior to final determination by a pathologist as a quality assurance procedure. The aim of this project was to evaluate the interobserver reproducibility of HER2/*neu* analysis among CTs and pathologists.

Design: HER2/*neu* was performed on 4234 paraffin-embedded breast tissue specimens using the DAKO HercepTest™ (DakoCytomation, Carpinteria, CA). One of 9 CTs classified each specimen as 0, 1+, 2+, or 3+ for HER-2/*neu* protein expression. One of 7 pathologists provided a final classification for each specimen. FISH analysis using PathVysion™ (Abbott Molecular, Inc., Des Plaines, IL) was performed on specimens classified as 2+. Only specimens having a CT score, a pathologist score, and a FISH result (if applicable) were analyzed in this study.

Results: Complete concordance between CT and pathologist results was established in 3532 (83%) of all cases. The majority of discordant cases were 1+ and 2+ cases. There were 1041 of 4234 (25%) specimens with a pathologist score of 2+ by IHC that were triaged for FISH analysis. The range of cases scored as 2+ between individual pathologists was 15-33% (mean 25%). Of these 1040 cases, 14% demonstrated HER-2/*neu* amplification (range 11-22%). The range of cases scored by CTs as 2+ was 23-39% (mean 31%). There were 171 discordant cases classified as 2+ by the pathologist; 60 cases were scored as 3+ by the CT (31/60 or 52% FISH amplified) and 111 cases were scored as 1+ by the CT (5/111 or 5% FISH amplified). One case was scored as 3+ by the CT and downgraded to 1+ by the pathologist.

Conclusions: This study demonstrates high interobserver reproducibility between CT and pathologist in the evaluation of IHC HER-2/*neu* results. CTs classified a higher percentage of 2+ cases. Additionally, over half of the cases downgraded by pathologists from 3+ to 2+ were FISH amplified and only 5% of those upgraded from 1+ to 2+ were FISH amplified, indicating that CTs are scoring specimens with acceptable accuracy. In summary, the data indicate that CTs provide reproducible and highly concordant results as the primary evaluators of IHC HER-2/*neu* specimens.

1548 Defining Specimen Mis-Identification by Molecular DNA Analysis

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Background: Assuring accurate patient and specimen identification is a national patient safety goal and the initial step in a laboratory test quality. Mis-identified cases in anatomic pathology (AP) are usually not actively sought by the laboratory, but rather, passively acquired when the clinician receives a nonsensical result. In AP, this frequency is thought to be in the order of 1 per 5,000 cases. However, the actual rate of mis-identification is unknown. We attempted to define that number by focusing on the larger testing volumes of the clinical laboratory where discrepant mean corpuscular hemoglobin volume (MCV) from samples on same patients are compared electronically (delta check) to flag potentially mis-identified blood specimens.

Design: We selected blood specimens that were flagged over 6 days by MCV delta checks at Henry Ford Hospital. Specimens differed by + 3 femtoliters in MCV from one also analyzed on the same patient and were evaluated by ABI Identifiler DNA kit to determine genetic identity.

Results: Of 4269 blood specimens tested on core lab hematology analyzers, 6 were delta flagged and rejected on lab review as mis-identified. An additional 151 (3.5%) were found questionable because of MCV delta check differences. Paired samples for the same individual were recovered for 92 of these from inpatient and emergency room patients (18 pre and 74 post). By molecular genotyping, 89 of 92 (97%) specimens had an identical genotype, confirming that the differences in MCV were likely due to therapy, blood transfusion, or other disease related reasons. Three samples (3.3%) had different genotypes, indicating they were from different individuals, and were mislabeled at phlebotomy. Thus, roughly 1 of 1000 cases submitted for complete blood count testing was mis-identified.

Conclusions: Evaluating blood specimens by DNA analysis provides a larger, more readily obtainable denominator to generate an estimate of actual specimen mis-identification that the laboratory inherits from the pre-analytical phase of clinical specimen collection and labeling. We find this mis-identification rate is 5 times greater than the previous estimate of AP and Blood Bank specimens thought to be mis-identified by virtue of non-sensical result (wrong tissue, blood type) or wrong patient (not biopsied). This study underscores the importance of investing in quality assurance efforts that include pre-analytic process standardization and integration of new technologies to assure maintenance of patient and specimen identity in laboratory testing.

1549 Critical Values in Pediatric Surgical Pathology: Policy Development, Process Implementation, and Reporting in a Children's Hospital

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Background: "Critical values" (CV) reporting is a standard practice in laboratory medicine. Recently the scope of CVs has been expanded to surgical and anatomic pathology by accrediting agencies for patient care and safety. The purpose of this project was to improve timeliness and quality of patient care by defining pediatric surgical pathology (PSP) CVs and documenting verbal reports.

Design: Pediatric medical and surgical specialists and pediatric pathologists were surveyed for potential PSP CVs. A CV list and conceptual, current condition, and target condition flow charts were developed. A standard operating procedure (SOP) was developed. Institutional pathologists were trained in the identification, reporting, and documentation of PSP CVs. Retrospective analysis of PSP CV reporting, prior to the SOP, and concurrent analysis following SOP implementation were studied.

Results: Surveys were completed by 26 physicians. A list of PSP CVs was based on survey results, with Medical Executive Committee approval. Retrospective review of selected diagnoses prior to the project revealed that 80% of PSP CV were documented as verbally reported (59% of new tumor diagnoses, 100% of graft versus host disease reports, 100% of major frozen section discrepancies, and 90% of rectal biopsies for suspected Hirschsprung disease). After SOP implementation, 97% of PSP CV were reported and documented. CV cases accounted for 9% of PSP accessions. The distribution of 210 CVs in a 6-month period after the SOP included tumor diagnoses (37%), invasive organisms (10%), possible Hirschsprung disease (10%), crescents in renal biopsies (2%), erythema multiforme/Stevens-Johnson syndrome (1%), a major discrepancy between preoperative and final diagnosis (1%), and other pathologic diagnoses with immediate implications for clinical management (38%). 14% of CVs involved transplant recipients. Positive feedback has been received from physicians and other healthcare professionals.

Conclusions: PSP CV reporting offers the potential for improved patient care through timely communication and provides an opportunity for systemwide use in pediatric care. These results demonstrate the utility of policy development and process implementation with participation by a multidisciplinary team. This study provides the first systematically derived CVs for PSP.

1550 PCR-Based Microsatellite Analysis of Surgical Specimen Identity: Seven Years of Clinical Application

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Background: Despite policies and practices designed to ensure accurate identity of surgical pathology specimens, errors do occur. The fallout of misidentifying a specimen or of contaminating one specimen with tissue from another can be devastating. PCR-based microsatellite analysis for identity testing has been extensively utilized in the field of forensic pathology, but its role as a tool in surgical pathology has not been adequately addressed. The purpose of this study was to analyze the utility of this technique as a quality assurance method based on a 7-year experience at a large referral hospital.

Design: Surgical pathology cases submitted for identity testing were identified through a search of the files of the Molecular Diagnostics Laboratory of The Johns Hopkins Medical Institutions. Clinical, pathological and genetic identity data were obtained from the Surgical Pathology and Molecular Pathology databases.

Results: Forty-one surgical pathology cases submitted for tissue identity testing were identified during a 7-year period. There was a trend toward increasing utilization from 1 case in 1999 to 11 cases from 1/1/2006 to present. 23 (56%) cases were specimens obtained from other hospitals. A variety of organ sites were represented including prostate (n=17), upper GI (n=6), lower GI (n=5), breast (n=2), kidney (n=2), larynx (n=2), and other sites (n=7). 36 (88%) cases were biopsies and 5 (12%) were resections with or without a pre-operative biopsy. Tissue identity analysis was requested to either confirm a match between the specimen and patient (n=19, 46%) or to confirm a match between a tissue fragment and the rest of a specimen (i.e. rule out "floater") (n=22, 54%). Molecular identity testing established non-identity in 16 (39%) cases. In all of these cases (n=16, 100%) documentation of non-identity had a dramatic impact on the diagnosis. For example, in half of the tested prostate biopsies that showed an adenocarcinoma in a core fragment, the involved core was found to be a contamination from another patient.

Conclusions: PCR-based genetic strategies can establish tissue identity in a way that routine microscopy cannot. The ability to do so can uncover subtle errors that may dramatically alter patient management. Utilization of molecular identity testing should be considered when mislabeling of a diagnostic specimen is suspected (e.g. disparity between the clinical picture and pathologic findings) or when the possibility of contamination from another specimen could affect patient prognosis and management.

1551 Defining the Magnitude of Internal Process Defects in Surgical Pathology

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Background: The frequency of process defects encountered in the analytic phase of Surgical Pathology (SP) from point of specimen receipt to final report transmission is not known. The literature based on amended pathology reports does not address these internal defects that may be repaired if detected but are not often recorded. Knowledge of these defects and how they arise in the mostly manual processes of SP is key to planning quality improvements.

Design: To assess types of internal defects, we surveyed professional, technical and secretarial staff in the division of SP at Henry Ford Hospital (annual accession volume 48,000 cases). From the staff poll, we defined the top 10 defects commonly encountered